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CONTENTS

		PAGE
GUTHRIE, M. J., AND HIBBARD, H.	*Cleavage and Mesenchyme Formation in Toxopneustes variegatus*	139
MOORE, E. LUCILE.	*The Effect of Adrenin upon the Rate of Locomotion of Planaria and of Toad Larvæ*	157
SEYSTER, E. W.	*Eye Facet Number as Influenced by Temperature in the Bareyed Mutant of Drosophila melanogaster (Ampelophila)*	168
KRECKER, F. H.	*Circulation of the Cœlomic Fluid in a Nematode*	183
BUDINGTON, R. A.	*Influence of Certain Ductless Gland Substances on the Growth of Plant Tissues*	188
TANNREUTHER, G. W.	*Studies on the Rotifer Asplanchnia ebbesbornii, with Special Reference to the Male*	194

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BIOLOGICAL BULLETIN

CLEAVAGE AND MESENCHYME FORMATION IN TOXOPNEUSTES VARIEGATUS.

MARY J. GUTHRIE AND HOPE HIBBARD,
BRYN MAWR COLLEGE.

This investigation was undertaken for the purpose of determining the origin of the primary mesenchyme in Echinoidea. Although numerous researches have been carried out on the early stages in the development of echinoids, the question of the origin of the mesenchyme has never been definitely settled.

MacBride,[1] writing of *Echinus esculentus*, states that "this mesenchyme originates, almost certainly, from the descendants of the smaller micromeres." The figures which Boveri (1901) gives of the eggs of *Strongylocentrotus lividus* indicate that this is true, and it is believed that unmistakable evidence is herein presented that in *Toxopneustes variegatus* (*Lytechinus variegatus*) the mesenchyme arises from the micromeres.

MATERIAL AND METHODS.

The material on which this investigation was carried out was obtained by Dr. D. H. Tennent, during the summer of 1908, in the course of work at the United States Fisheries Biological Station, Beaufort, N. C.

The segmenting eggs were fixed in either picro-acetic or sublimate-acetic. In both cases the solutions were two per cent. acetic acid. The observations recorded here were made on whole embryos stained with borax carmine and safranin, and on sectioned material stained with borax carmine or iron hæmatoxylin.

In addition to the above material, Dr. Tennent lent camera

[1] "Text-book of Embryology," p. 507.

lucida sketches of the cleavage stages which he made from living eggs. While it is possible to make out these stages in the preserved specimens, they are much clearer in the living eggs; and his sketches have been used to illustrate the segmentation changes.

Early Cleavage.

The *Toxopneustes* egg has been found especially favorable for many kinds of study because of its transparency and beautiful clearness of structure, when fixed as well as while alive.

As this paper deals with a definitely limited phase of development it seems best not to include a detailed description of the egg, or its jelly layer, or of the manner of formation of the fertilization membrane, but to pass at once to the consideration of cleavage.

The first cleavage of the *Toxopneustes* egg is vertical and results in two equal cells being formed about forty minutes after insemination (Figs. 1, 2, 3 and 4). Tennent (1911) gave the characteristic rhythm for *Toxopneustes* cleavage at 28° C., and the time intervals indicated are from his paper. This rhythm may be altered by changes in temperature as has been shown for example by Loeb and Chamberlain (1915) who worked with *Arbacia* eggs. They found a close correlation between the temperature of the water in which the eggs were kept and the period of time elapsing between insemination and the first cleavage, and were, therefore, able to calculate the temperature coefficient for cell division at varying temperatures.

The second cleavage, about one hour and ten minutes after insemination, is also vertical, and divides the egg into four equal cells which are shown in polar view in Fig. 5. After one hour and thirty-five minutes, the third cleavage furrow coming in horizontally divides the egg into eight equal cells (Figs. 6 and 7). This is the last cleavage in which all of the cells are equally divided.

A twelve cell stage is formed by a horizontal division which cuts off four small cells, the first set of micromeres, at the vegetal pole. Practically coincident with this, the four cells at the animal pole are divided by vertical furrows into eight. In the sixteen cell stage there are therefore, three different kinds of

cells as regards size, these being respectively, the small macromeres, a group of eight medium-sized cells at the animal pole; the micrometers, four small cells at the vegetal pole; and the large macromeres, four large cells lying between the two groups just mentioned. These are shown as seen from the side in Fig. 8 and as seen from the vegetal pole in Fig. 9. It is one hour and fifty minutes after the addition of the sperm when this stage is complete.

From this time on, all of the cells do not divide simultaneously. Two hours and ten minutes after insemination the four large macromeres are divided vertically into eight, and the eight small macromeres are divided vertically into sixteen. About ten minutes later the micromeres are cut horizontally into eight, but this is an unequal division, the four smaller micromeres lying nearer the vegetal pole (Fig. 10). The completed thirty-two-celled embryo is shown from the side in Fig. 11, and the crowding in of the micromeres can be noticed at this stage.

The next furrow cuts the lower set of eight large macromeres horizontally, and twenty minutes later, or two hours and fifty-five minutes after insemination, vertical cleavages divide the upper set of large macromeres into sixteen, and the sixteen small macromeres into thirty-two. After three hours and five minutes, the upper micromeres are divided vertically into eight, the four micromeres referred to above as lying nearer the vegetal pole, now being crowded back and partially concealed by this ring of eight cells. Five minutes later the lowest set of macromeres is divided by vertical cleavages into sixteen (Fig. 12). Twelve of the seventy-six cells of this stage are micromeres, the later development of which will be taken up presently.

The following table (page 142) shows in a diagrammatic way, the sequence of cleavage in the *Toxopneustes* egg. The time indicated is the elapsed time after insemination.

Theel's (1892) account for *Echinocyamus pusillus* differs from this in that he describes the micromeres as being cut off at the animal pole. According to his description the sixteen-cell stage is completed in about two hours and twenty minutes and is accomplished as follows: "First the four upper segments are divided by a horizontal plane into four small cells or micromeres and

TABLE SHOWING SEQUENCE OF CLEAVAGE.

Time.	40 m.	1 h. 10 m.	1 h. 35 m.	1 h. 50 m.	1 h. 50 m.	2 h. 10 m.	2 h. 20 m.	2 h. 35 m.	2 h. 55 m.	2 h. 55 m.	3 h. 5 m.	3 h. 10 m.
Small macromeres			4	4	8	16	16	16	32	32	32	32
Blastomeres	2	4										
Large macromeres			4	4	4	8	8	16	16	24	24	32
Micromeres				4	4	4	4	4	4	4	8	8
							4	4	4	4	4	4
Total cells	2	4	8	12	16	28	32	40	56	64	68	76

[1] It will be noticed that there are two columns headed 1 h. 50 m. and two headed 2 h. 55 m. This is for the purpose of indicating that the formation of the micromeres is completed before complete division of the four small macromeres, about one hour and fifty minutes after insemination, and that the division of the sixteen small macromeres is completed before that of the upper eight large macromeres, two hours and fifty-five minutes after insemination. The duration of an actual twelve cell and of an actual fifty-six-cell stage is short.

four large ones. Immediately after this segmentation or, though more seldom, before it is accomplished, the four lower segments are divided by a vertical plane into eight almost equal spheres, which become arranged in a more or less irregular manner in two rows."

Further, in *Echinocyamus*, two hours and thirty-five minutes after fecundation, the large macromeres are divided vertically, the small macromeres are cut horizontally, and after fifteen or twenty minutes "the four micromeres at the animal pole become parted by horizontal planes into eight, four of which are smaller, contain a clearer protoplasm and, as they lie in the same plane, constitute the animal pole itself; the four remaining ones have their place somewhat outside and below." It will be noticed that the small macromeres are divided horizontally in *Echinocyamus*, while in *Toxopneustes* the division is vertical.

Theel fell into error concerning the micromeres probably because he assumed that they were homologous with the micromeres of flatworms, annelids and molluscs. Boveri ('01) in his study of the egg of *Strongylocentrotus lividus*, demonstrated that it is at the vegetal pole that the micromeres are separated at the twelve-cell stage.

FORMATION OF MESENCHYME.

Very early in the development of the *Toxopneustes* blastula the cells which are to give rise to the future mesenchyme can

be distinguished. With the cutting off of the micromeres there is separated from the rest of the embryo that protoplasm which later goes to form the mesenchyme. These cells, or cells derived from them, must pass from the wall of the blastula into its cavity; and as early as the sixteen-celled stage some of the first four micromeres exhibit this tendency to migrate inward. Sections passing through the poles of a sixteen-celled stage show the micromeres visibly crowded. Figs. 13 and 14 are sections of two different individuals in this stage, and the micromeres at the vegetal pole are projecting slightly into the blastocœle with their inner ends bulging as a result of pressure from the adjacent cells in the wall. The difference in the number of the micromeres in the two figures is due to the planes of the sections. Fig. 13 is cut parallel to the short axis of the group of micromeres, while Fig. 14 is cut parallel to the long axis of the group.

A later stage is shown in Fig. 15 which is a somewhat oblique section through the micromeres. In this case the micromeres have multiplied and the process of pushing in has continued until one cell of the particular section in question has lost all connection with the surface.

Figs. 16*a*, 16*b* and 16*c* represent three serial sections of one specimen which is slightly older than the preceding one. Here the migration is quite evident. From this time on the micromeres are not readily distinguishable by their small size as heretofore, since by an unequal rate of division, all the cells have become more nearly the same size. The fact that in earlier stages it was the micromeres, and they alone, which passed inward, and the fact that throughout the stages the migration is restricted to a limited area in the wall, afford proof that in the later stages the mass of cells which has passed inward represents the offspring of the original micromeres.

A more pronounced wandering in of cells is shown in Figs. 17*a*, 17*b* and 17*c*—serial sections of one embryo. Here, certainly, pressure from without has ceased to operate as the cause of migration, for the wandering cells are free to a great degree from contact with cells of the wall.

There have been various theories of the cause of the separation of the mesenchyme cells from the wall of the blastula in other

forms. Grave, in *Ophiura brevispina*, describes the mesenchyme cells as being pushed or squeezed inward by the coercive pressure of the organism as a whole, though this is not the whole story of the process. MacBride describes the migration of mesenchyme cells in *Asterias vulgaris* thus: "The 'wandering' of these cells seems to be effected by their emitting long filamentous pseudopodia which span the blastocœle, and along these strands the body of the cell glides like a drop of dew on a spider's web."

Serial sections through an embryo figured in 18*a*, 18*b* and 18*c*, show the mesenchyme cells much more numerous, and those most recently budded off are compactly pressed together. In Fig. 19 the cells have multiplied still more and the mesenchyme lies close to that part of the wall from which it arose. Fig. 20, a stage still later, shows the mass of mesenchyme cells much larger but still compact. In this set of material this condition was found one hour and forty minutes before the first indication of gastrulation, namely, the flattening of the vegetal pole.

When the process of gastrulation begins, the mesenchyme cells migrate laterally and become arranged in a ring in the blastocœle about the polar axis of the embryo. The invagination of the archenteron proceeds from the vegetal pole toward the space unoccupied by mesenchyme. Fig. 21 is a very early gastrula with the mesenchyme cells lateral to the invaginating archenteron. A horizontal section through an early gastrula in approximately the same stage as Fig. 21 is shown in Fig. 22. The ring of mesenchyme is distinctly visible around the archenteron. A later gastrula is shown in Fig. 23 in which the secondary mesenchyme is being proliferated from the inner end of the archenteron. The primary mesenchyme may be seen at the sides of the archenteron. This specimen was somewhat distorted in sectioning and for comparison, Fig. 24, drawn from a camera lucida sketch of the living gastrula, has been inserted.

Since the publication of Theel's paper, in which the literature on the subject up to 1892 was discussed, the origin of the mesenchyme has been described for several echinoderms. Like the earlier accounts, these, with the exception of those of Boveri and MacBride, deal rather with the general question as to whether the mesenchyme arises in the blastula at the vegetal pole, or in

the gastrula from the archenteron, either during or after its invagination, than with the idea of associating the primary mesenchyme with an early differentiation in the segmenting egg.

The following table gives a resumé of the more recent literature, with the inclusion of Bury's observations of earlier date on *Antedon*.

TABLE SHOWING TIME OF MESENCHYME FORMATION.

Date.	Author.	Form.	Time of Mesenchyme Formation.
		ASTEROIDEA.	
1892	Field.	*Asterias vulgaris.*	Blastula, through gastrulation.
1896	MacBride.	*Asterina gibbosa.*	After complete gastrulation.
1902	Masterman.	*Cribrella oculata.*	After complete gastrulation.
1912	Gemmill.	*Solaster endeca.*	As morula changes to blastula.
1914	Gemmill.	*Asterias rubens.*	Late gastrula.
		OPHIUROIDEA.	
1897	MacBride.	*Ophiothrix fragilis.*	Blastula.
1916	Grave.	*Ophiura brevispina.*	Blastula.
		ECHINOIDEA.	
1901	Boveri.	*Strongylocentrotus lividus.*	Blastula.
1903	MacBride.	*Echinus esculentus.*	Blastula.
		" *miliaris.*	Blastula.
		" *acutus.*	Blastula.
1904	Schmidt.	*Echinus microtuberculatus.*	Blastula.
1914	MacBride.	*Echinocardium cordatum.*	Blastula.
1914	Tennent.	*Cidaris tribuloides.*	Gastrula.
		CRINOIDEA AND HOLOTHUROIDEA.	
1898	Clark.	*Synapta vivipara.*	Gastrula.
1909	Edwards.	*Holothuria floridana*	Blastula at beginning gastrulation.
1888	Bury.	*Antedon rosacea.*	Gastrula.
1892	Seeliger.	" "	Gastrula.

It will be seen that among the Asteroidea there are some differences between various forms in their methods of mesenchyme formation. Field (1892) described these cells in *Asterias vulgaris* as being given off from the developing archenteron even from the beginning of its invagination. Gemmill (1914) found no mesenchyme cells in the normal larvæ of *Asterias rubens* until gastrulation was well advanced, when they appeared at the end of the archenteron. MacBride (1896) found in *Asterina gibbosa* that no mesenchyme was formed until after gastrulation was complete. In connection with these accounts it is interesting to note the results of Herbst (1896) who experimentally induced larvæ of *Asterias glacialis*, which normally produce mesenchyme only

after gastrulation, to form large numbers of mesenchyme cells while still in the blastula stage.

In contrast with these processes, Masterman (1902) in *Cribrella oculata*, and Gemmill (1912) in *Solaster endeca*, have described a very different series of events. In *Solaster* the cleavage results in a solid morula of nearly equal cells. The outside of this mass then becomes wrinkled by the appearance of convoluted furrows which so increase the surface that the cells inside can become arranged in a single layer, leaving a flattened, irregular cavity, the blastocœle. The furrows gradually disappear as the cells over the entire surface become longer and narrower until the larva becomes smooth once more. The initiation of the process of gastrulation also aids in flattening the surface. During these changes some wandering cells fail to arrange themselves in a layer with the others and are left behind in the cavity of the blastula. These are the primary mesenchyme cells. In *Cribrella* similar phenomena occur, but instead of being left behind as the morula changes to a blastula, the mesenchyme cells are budded off after invagination, from the "hypoblast" into the blastocœle, in the manner of the more typical Asteroidea. In addition to this, cells morphologically similar to mesenchyme cells are budded off from the other side of the "hypoblast" into the archenteron, and were called "hypenchyme" by Masterman.

Even fewer species of the Ophiuroidea have been investigated on this point. Grave (1916) in *Ophiura brevispina* found a large number of mesenchyme cells in the blastocœle of early stages. MacBride (1897) observed mesenchyme in blastulæ of *Ophiothrix fragilis* between four and five hours after fertilization, while gastrulation did not begin until about the eighteenth hour.

The majority of Echinoidea show early mesenchyme formation. MacBride (1903) figured blastulæ of *Echinus miliaris*, *E. acutus* and *E. esculentus* with mesenchyme forming at one end, which was, in some cases, slightly flattened. In *E. esculentus* he found about fifty mesenchyme cells which were probably derived from the micromeres in the blastula. In *Echinocardium cordatum* (MacBride 1914) mesenchyme is found at the vegetal pole long before any signs of gastrulation. MacBride was unable to confirm the observations of Fleischman (1888) on this form with

regard to the four arch-mesenchyme cells which Fleischman found. Schmidt (1904) observed mesenchyme cells in the blastula of *Echinus microtuberculatus*. They were present at the seventeen-hour stage and continued to be formed until the twenty-four hour stage. Boveri (1901) in his work on *Strongylocentrotus lividus*, found in the unsegmented egg a portion at the vegetal pole that was unpigmented. This region gave rise to the micromeres, and they in turn to mesenchyme in the blastula stage. The lack of pigment in these cells made their development easy to follow.

Tennent (1911) noted the presence of four mesenchyme cells in the blastocœle of the *Toxopneustes* blastula about eight hours after the fertilization of the egg and about an hour before the beginning of gastrulation. An exception to the typical echinoid condition is shown by *Cidaris tribuloides* (Tennent, 1914), in which the formation of the mesenchyme is delayed and is given off from the inner end of the archenteron in the gastrula.

To our knowledge of mesenchyme formation in the Holothuroidea the observations of Clark (1898) that in the blastula of *Synapta* there is no evidence of mesenchyme cells, but that they arise later, wandering in from the whole surface of the archenteron, and of Edwards (1909) that in *Holothuria floridana* the mesenchyme begins by cell-proliferation at the vegetative pole of the blastula, while at the same time gastrulation takes place, confirm the earlier evidence of Korschelt, Selenka and Ludwig to the effect that in the Holothuroidea the time of mesenchyme formation varies.

Bury (1888) and Seeliger (1892) from their study of the development of *Antedon rosacea* agree that the formation of the mesenchyme does not begin until after the beginning of the processes of gastrulation.

From these accounts it may be seen that, with the exception of Boveri's work on *Strongylocentrotus* and MacBride's work on *Echinus esculentus*, little attempt has been made to trace the origin of the mesenchyme cells further back than to the approximate point in the larva from which they arise. If formed early they come from the vegetal pole of the blastula, if late from the archenteron. Theel makes the statement that "the mesenchyme

arises earliest, with very few exceptions, in those groups of echinoderms in which the larvæ have their skeleton earliest developed." Since one of the functions of the primary mesenchyme is the formation of the skeletal rods of the larvæ the reason for this correlation is evident.

In this paper it has been shown that the primary mesenchyme in *Toxopneustes* comes from the micromeres. Driesch (1900) has shown by experiments on fragments of blastulæ, that after the micromeres have been formed, the cells at the opposite pole of the larva are unable to form mesenchyme. The micromeres arise at the vegetal pole of the segmenting egg, but cannot be considered as either ectoderm or endoderm. The primary mesenchyme is the result of an earlier differentiation than either ectoderm or endoderm.

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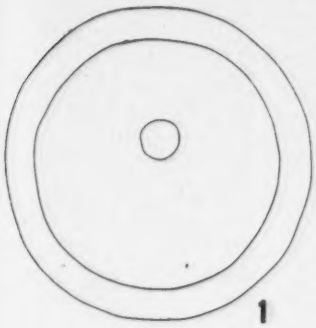
DESCRIPTION OF PLATES.

Figs. 1 to 12 and Fig. 24 were made from camera lucida sketches of living eggs. A pantograph was used to enlarge them two and one half times, and this gave a magnification of 1,050 diameters. They have been reduced one half in reproduction so that the magnification is now 525 diameters.

Figs. 13 to 23 were made from camera lucida drawings of mounted sections which were enlarged two and one half times and reduced one half giving a final magnification of 725 diameters.

EXPLANATION OF PLATE I.

FIG. 1. Fertilized egg.
FIG. 2. Incomplete 2-celled stage.
FIGS. 3 and 4. 2-celled stage.
FIG. 5. Polar view of 4-celled stage.
FIG. 6. Incomplete 8-celled stage.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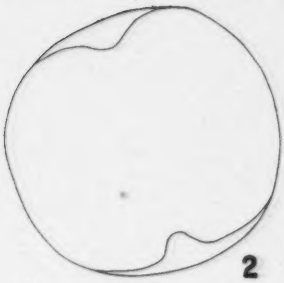
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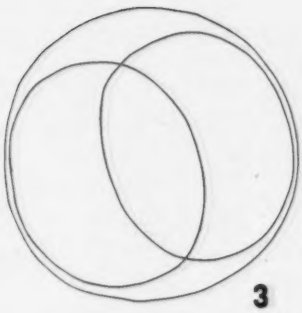
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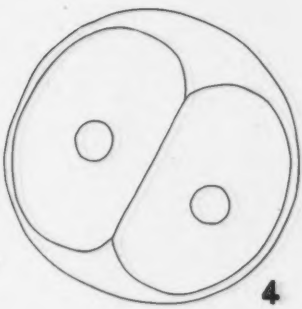
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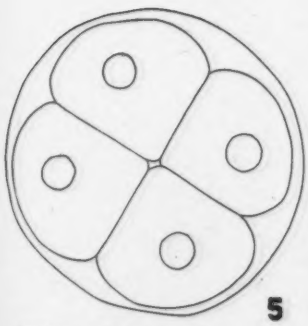
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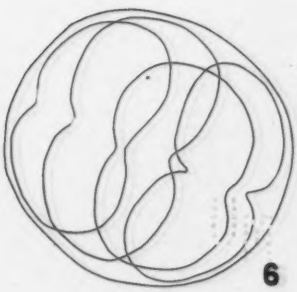
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GUTHRIE AND HIBBARD.

EXPLANATION OF PLATE II.

FIG. 7. Side view of 8-celled stage.

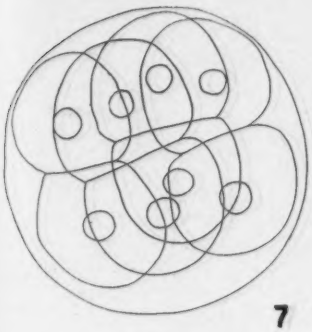
FIG. 8. Side view of 16-celled stage showing the micromeres cut off at the vegetal pole.

FIG. 9. View of 16-celled stage from vegetal pole.

FIG. 10. View of 32-celled stage from vegetal pole showing second set of micromeres.

FIG. 11. Side view of 32-celled stage.

FIG. 12. Polar view of a 76-celled stage showing the twelve micromeres.



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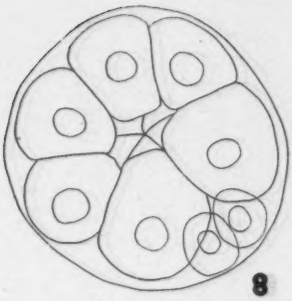

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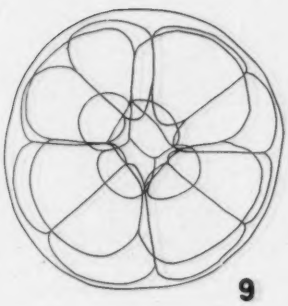

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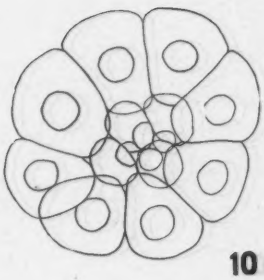

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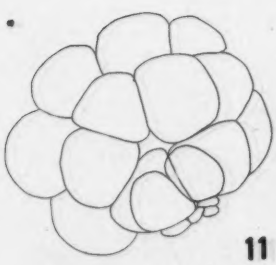

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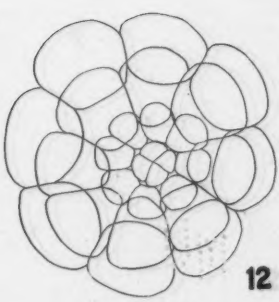

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EXPLANATION OF PLATE III.

FIG. 13. Section through a 16-celled stage passing through the short axis of the group of micromeres.

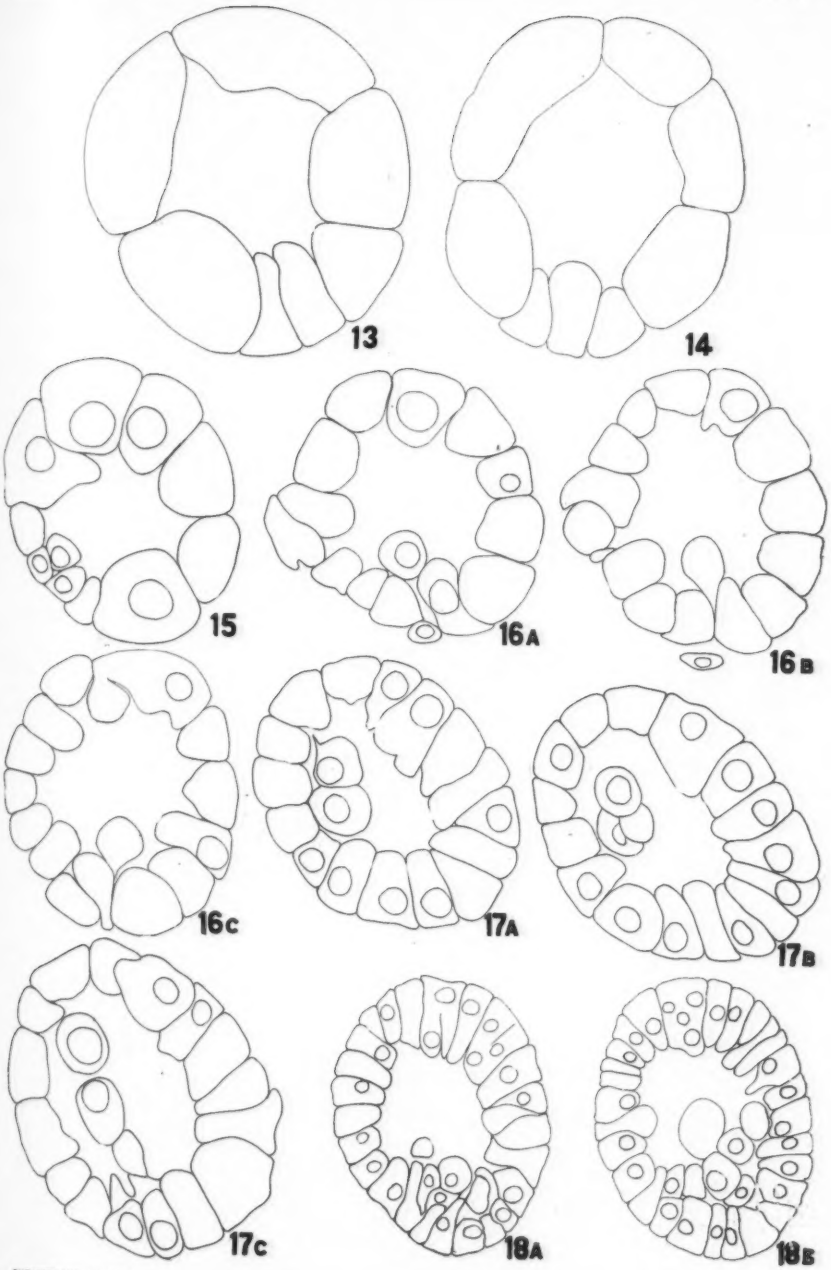
FIG. 14. Section through a 16-celled stage passing through the long axis of the group of micromeres.

FIG. 15. Section of slightly older stage showing multiplication of micromeres.

FIGS. 16*a*, 16*b*, 16*c*. Serial sections of an older embryo showing migration of micromeres.

FIGS. 17*a*, 17*b*, 17*c*. Serial sections of a still older embryo showing further migration of micromeres.

FIGS. 18*a*, 18*b*. Two of a series of sections showing more numerous mesenchyme cells (micromeres).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15
16A
16B
16C
17A
17B
17C
18A
18B

EXPLANATION OF PLATE IV.

FIG. 18*c*. Third section in the same series as Figs. 18*a* and 18*b* showing more numerous and compact mesenchyme cells.

FIG. 19. Section through slightly older embryo showing continued multiplication of mesenchyme cells.

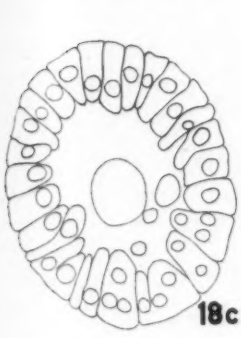
FIG. 20. Section through a later stage than Fig. 19.

FIG. 21. Vertical section through an early gastrula.

FIG. 22. Horizontal section of same stage as Fig. 21.

FIG. 23. Vertical section of older gastrula.

FIG. 24. Entire living gastrula.



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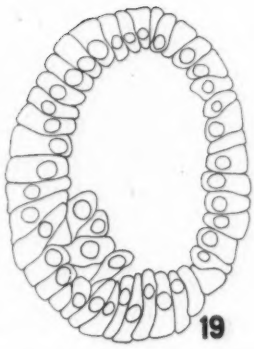

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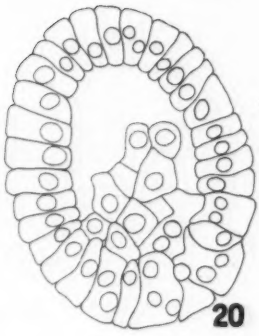

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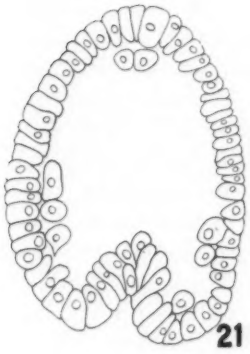

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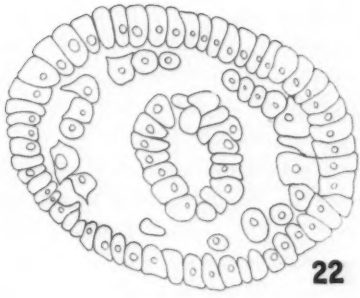

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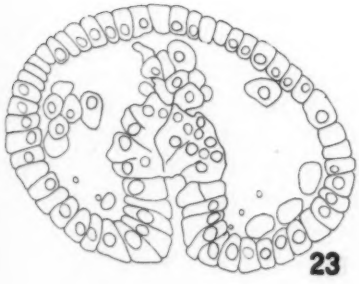

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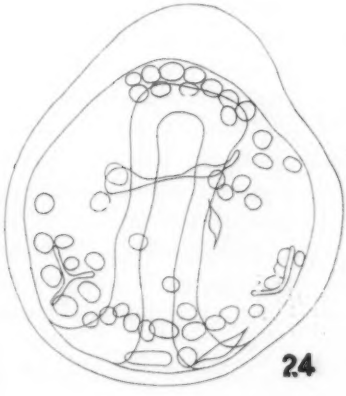

24

THE EFFECT OF ADRENIN UPON THE RATE OF LOCOMOTION OF PLANARIA AND OF TOAD LARVÆ.

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In his investigation of the chromaffine system of annelids, Gaskell (2) has pointed out the close morphological and physiological relationship which exists between the chromaffine, the sympathetic and the contractile vascular systems, wherever these are found. He holds that the presence of any systems corresponding physiologically to the first two, necessarily implies the presence of the third on which to act; and in support of this view, he finds in the annelid group, in those forms which possess a contractile vascular system, a cell which he considers the common ancestor of the sympathetic and chromaffine systems. If, therefore, the earliest indication of chromaffine tissue is found among the annelida, it may be safely assumed that in the platyhelminth group in which there is no contractile vascular system, there is nothing comparable to the adrenin-producing cells of higher forms. The effect of adrenal extract is not, however, confined to the regulation of the cardio-vascular apparatus, although this seems to be one of its chief functions. Elliott (1) has definitely shown that it affects other forms of smooth muscle in a manner similar to the sympathetic nervous system, causing either an excitation or an inhibition, according to the nature of the sympathetic fibers supplying the tissue. Its influence is, furthermore, not even limited to smooth muscle for skeletal muscle is affected, also, as various investigators have demonstrated. Is it possible, then, that this secretion of the adrenal medulla exercises an influence upon the contracility of the simple muscles of an organism such as *Planaria*, which does not itself manufacture adrenin?

Bands of circular, longitudinal and diagonal muscles are found in the body wall of planarians; and it is by means of these that

locomotion is made possible. It had been generally believed that only the crawling movement is accomplished through muscular contractions, and that gliding results chiefly from the beating of ventral cilia; but Stringer (8) has shown that when muscular activity is entirely checked locomotion does not occur, even though the cilia are beating vigorously; and she concludes that the "locomotion of planarians is essentially a muscular act in which the cilia play no necessary part."

Although the influence of adrenin upon the muscular contractility of *Planaria* cannot be exactly homologized with that upon vertebrate muscle, it is well to bear in mind the results of these investigations. Oliver, and Schäfer (7) report both a greater and a more prolonged contraction of the gastrocnemius in frogs as the result of adrenin injection, and also an increase in muscular activity following administration of the extract by mouth. Takajasu (9), however, working upon isolated muscle, obtained just the opposite effect. He found that the curve of contraction in skeletal muscle, immersed in solutions of adrenin, varying from 1 : 500,000 to 1 : 5,000, becomes successively lower and of shorter duration, although there occurs a preliminary period during which the contractions are slightly prolonged. Gruber (3 and 4) has obtained interesting results with muscle preparations in which fatigue has been induced either electrically or chemically. In these cases, adrenin, instead of causing a still further lowering of the fatigue curve, really produces a rapid recovery of the normal irritability of the muscle. Unlike Oliver and Schäfer, however, he reports no improvement in muscles not fatigued. These investigators, it is evident, have employed various means of introducing the extract, and have worked upon both entire animals and isolated muscle. With planarians, injection is impracticable, and the immersion of isolated muscle altogether impossible; but by placing entire individuals in solutions of adrenin, a combination of the methods of immersion and feeding is obtained.

The experiments here described were carried out upon *Planaria doratocephala*, and *Planaria velata*. The worms were placed in large finger bowls, containing spring water, and kept in the laboratory at as nearly constant temperature as possible. Food

consisting of finely minced earthworms of raw meat, was supplied two or three times a week, and at each feeding the water was changed. The Parke, Davis, and Company preparation of adrenalin chloride[1] was at first used; but it was found unsatisfactory, as animals immersed in solutions varying from 1 : 1,000 to 1 : 20,000 soon became inactive, and died within less than an hour. Solutions of the same strength, however, prepared from adrenin in tablet or in crystal form did not produce the same effect; and it seemed not improbable that it was the chloretone in the first preparation which caused the rapid loss of activity. To test this hypothesis, a solution of chloretone was prepared, equal in strength to that contained in the adrenalin chloride preparation, and a comparison was made of the effects upon *Planaria* of similar dilutions of these two. In both cases complete loss of locomotor power quickly resulted. The chloretone alone (1 : 2,000) was sufficient to cause within an hour, a noticeable elongation and lack of response to mechanical stimuli, an effect which is not surprising, since chloretone of 1 : 1,000 strength is recommended as a ready anæsthetic for planarians (10).

In the following experiments, therefore, adrenin in tablet and in crystal form was used. In order to determine whether or not this extract exercises any influence whatever upon *Planaria*, active individuals were put into small bottles containing solutions of adrenin of different strengths, and subjected to the influence of the extract for varying periods of time. It was necessary to keep the bottles corked to prevent the rapid oxidation of the adrenin, but there was enough oxygen present to sustain the worms; and the fact that planarians in corked bottles containing water only were not affected seems proof that the results noted cannot be attributed to lack of oxygen. In the preliminary experiments it was found that animals in solutions of adrenin, varying from 1 : 1,000 to 1 : 15,000 died in less than three days, but in more dilute solutions they remained alive and active even after a week. Most of the later experiments were, therefore, made with a 1 : 15,000 solution, for one day, or with stronger solutions for a shorter period.

[1] Each fluid ounce of this preparation contains $\frac{2}{3}$ grain of adrenalin chloride and $2\frac{1}{4}$ grains of chloretone.

It was difficult to find a means of measuring the influence of the extract upon the musculature. After unsuccessful attempts to record the effect through electrical stimulation, a method of testing muscular activity by means of the rate of locomotion was adopted. A narrow trough, 6.5 cm. long by .3 cm. in width, was made by cementing two ordinary glass slides, side by side, upon a glass plate. Planarians, put into the trough with a little water, readily progressed from one end to the other, usually in the angle formed by the bottom plate and the edge of one slide. A starting place was then marked off; and with a stop-watch the rate of travel was measured. Only continuous non-stop journeys, from the time the head passed the starting point until it reached the end of the trough, were recorded. A large variation was found both in successive rates of the same individual, and in rates of different normal individuals; but by taking the average of a number of successive rates for each animal, and by using the same ones both before and after treatment with adrenin, it was possible to secure results which seem to be significant.

The animals were handled as far as possible in the same way in each experiment; and to avoid differences due to the influence of light, the trough was placed on the table in such a position that the animals moved away from the window in a direction parallel with the entering rays of light. Parker and Burnett (6) have shown that planarians have a "tendency to turn away from the course when directed toward the source of light," and this was easily verified, as it was found almost impossible to keep a worm traveling toward the window. In eighteen successive trials only once did an individual finish the whole length toward the light without reversing; but in the other direction full trips were readily obtained.

In testing the effect upon locomotion, the normal rates of the planarians in water were first recorded, then the rates immediately after exposure to the influence of adrenin, and finally the rates after recovery in water. In the first series (Table I.) ten individuals were used. Only two rates for each were taken under each of the three conditions; but a comparison of the averages of the twenty rates thus obtained indicates an effective influence of adrenin upon the locomotor activity. The average

normal rate was 53.4 seconds; but after one day in adrenin solution (1 : 15,000) the average time needed to travel the same distance was increased to 69.5 seconds. Furthermore, as an indication that the change was in response to a definite stimulation, and not merely the expression of a generally weakened physiological condition, such as does occur after a time when the animals are kept in the laboratory, there was a decided improvement after the animals had been placed in water again for two days. The rate this time was even better than the original, 51 seconds.

This same retarding influence of adrenin, followed by recovery in water was found in the next five planarians tested. But the third Series, as is shown in Table I., gave apparently very disappointing results. It may be interesting, however, to note that the animals of Groups B and C which gave good initial rates had been tested before with adrenin. The very poor rates, therefore, of the planarians of Groups A and D, which had been kept for some time in the laboratory, and had not been previously used, indicate that these animals were no longer in the same condition as those of the first experiments and might well be affected differently by the same extract.

Fresh planarians were then obtained and the same tests repeated. The results of these, also recorded in Table I., seem to confirm the conclusion drawn from the first experiments, that adrenin produces a decrease in the normal rate of locomotion of *Planaria*, which is overcome when the animals are again placed in water.

The nature of the effect of adrenin upon the muscular contraction of *Planaria* is not, however, as simple as the decrease in the rate of locomotion would indicate. Observation of the animals reveals clearly that the effect is not merely one of weakened muscular response to mechanical and contact stimuli. There seems to be no delay in the time of reaction to stimulation, and no constant decrease in the strength of the contraction. It is moreover, apparently not the same effect as that which Moore (5) found produced by strychnine, as no reversal of the normal response to stimulation was observed at any time after treatment with adrenin. Experimental animals, touched with a camel's

hair brush, reacted in the usual manner by contracting the longitudinal and relaxing the circular and transverse muscles. The effect was rather one of excitement, confined chiefly to the anterior end of the body, which seemed to make coördinated

TABLE I.

Series.	Strength of Sol.	Number of Animals.	Time Exposed to Sol.	No. of Rates Taken.	Average Initial Rate in Sec.	Average Rate in Adrenin.	Average Rate After Return to Water, 1–3 Days.
1.	1 : 15,000	10	24 hrs.	2	53.4	69.5	51
2.	1 : 15,000	5	19 hrs.	2	about 55.	84	59
3. A	1 : 15,000	4	24 hrs.	2	126.5	86.6	66.6
B	1 : 15,000	3	24 "	2	59.2	73.8	64.2
C	1 : 15,000	3	24 "	2	51.5	47.5	60.8
D	1 : 15,000	2	24 "	2	187.	85.5	103.5
E	1 : 15,000	3	22 "	5	69.	67.3	——
4. A	1 : 2,000	1	15 min.	5	91.8	139.8	131
B	1 : 2,000	1	20 "	5	71.6	206	137.5
C	1 : 20,000	3	30 "	5	41.1	48.2	37.2
D	1 : 10,000	4	20 "	5	33.1	52.2	44.3
E	1 : 10,000	4	20 "	5	33.1	51.2	41
F	1 : 10,000	2	20 "	5	34.8	50.9	30.4
G	1 : 10,000	2	20 "	5	30.6	38.7	25.7

locomotion difficult. The animals expended their energy in excited movements of the head, and occasionally were unable even to proceed the whole length of the track. When continuous trips were obtained, a decrease in rate was found, as has been noted; but this cannot be entirely due to a decrease in muscular activity, as there is frequently clear evidence of an increased irritability. At other times, however, the excitatory effect was not noted, and the slower rate was really indicative of a slower rhythmic contraction as the animal progressed along the trough.

This variability of result, expressed as it is in both cases by a decrease in rate, is difficult to explain; and it may be that an explanation is superfluous, and adrenin is not specific in its influence upon planarian muscle. It is possible, however, that we are dealing here with a definite action of the extract; and that a more detailed study of the effect upon the three kinds of muscles, and a more accurate determination of the physiological condition of the individuals at the time they are tested may throw more light on the subject. A possible explanation may be found in the duration of the influence of the extract. The

animals which showed to a greater extent the symptoms of excitement were those which had been treated with adrenin for an hour or less. Those which were in the solution for a day, with the exception of some of the groups of Table I., Series 3, showed almost uniformly a real slowing of the rate of muscular contraction. It may be that the initial effect produced by adrenin upon the muscular apparatus of planarians is excitatory in nature, and that the increased irritability, expressed in useless and non-coördinated movements really results in a decrease in the rate of locomotion. As the animals are left in the solution, however, the exciting effect wears off, and there results either an approximate return to normal, as in Series 3, Groups C and E, or a decrease in irritability, following naturally the undue excitement created at first.

This hypothesis of an initial excitatory effect might account also, for some of the other discrepancies of Series 3. The animals of Groups A and D, which showed such a poor initial rate in water may have been in a condition of lowered vitality, perhaps a state of fatigue. Here, as the results show, there followed an increased rate after treatment with adrenin. Might it not be possible that the excitatory effect of the extract was, in these cases, merely sufficient to raise the muscular tone to normal, without producing any symptoms of over-excitation? It is not unlikely that in these animals the increase in the rate of locomotion indicates the real effect of adrenin, which in most of the other experiments is marked by the decrease in rate due to the useless movements of excitement. This possibility may be lessened, perhaps, by the fact that the animals of Group A continued to improve even more after return to water, while those of Group D fell back, indicating that other factors were at work; but this does not exclude the view that adrenin produces a temporary improvement which may or may not become more lasting, dependent upon these other factors.

In order to determine whether or not the same results are produced by repeated doses of adrenin, several further tests were made, the results of which are recorded in Table II. After each treatment with the extract, one to three days in water were allowed for recovery before the next tests were made.

Such variable results were obtained, however, that it is impossible to say whether there is a tendency for *Planaria* to become less sensitive to repeated doses or not. By looking over the percentages of decrease it may be seen that eleven of the twelve animals responded to a lesser degree to the second treatment than to the first; but there is one great exception, and there is, in addition, the result, not recorded in the table, of a third test made with the last series where the decrease was 47 per cent., less than the first but greater than the second. Such variation was to be expected, from the nature of the problem, since the difference in the rate of locomotion does not express the whole of the effect. Although these last experiments add nothing new to our knowledge of planarian reaction to adrenin, they do distinctly confirm the fact that adrenin produces a slowing of the rate of locomotion through an over-excitability which expends itself to no effect.

TABLE II.

Series.	Strength of Sol.	No. Animals Used.	Time Exposed to Sol., Min.	No. Rates Taken.	Ave. Initial Rate in Sec.	First Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.	Second Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.
1	1:10,000	4	20–30	5	40	56.15	40.3	34.8	45.4	30.4	33.2
2	1:10,000	3	25–45	5	29.46	47.35	60.7	33.8	43.8	29.6	38.4
3	1:10,000	1	20	5	45.2	43.7	−03.3	36.2	60	65	53
4	1:10,000	4	20	3	38.4	64.7	68	45.3	57.9	27	41.9

The innervation of the muscles of *Planaria* has not yet been worked out, but it is probable, from the simple character of the nervous system, that the action of adrenin is directly upon the muscle and not through the nerve endings. This point, however, could not be successfully tested. In an attempt to ascertain the rôle of the nervous system it seemed desirable to compare the reactions of the anterior ends of the animals with those of the brainless parts, when exposed to the same solutions of adrenin. A number of individuals were cut in two just posterior to the brain region, and the wounds allowed to close over. It was practically impossible, however, to obtain any satisfactory rates from these pieces even in water, as the small head ends

could not be kept in a straight path, and the larger posterior parts moved too slowly in the beginning for any effect of adrenin to be noticeable. It may be noted in this connection that Gruber (3) obtained the same results with denervated vertebrate muscle as with normal nerve-muscle preparations.

Before concluding it may be of interest to mention the results of a few experiments upon toad larvæ. Only fifteen tadpoles were used; and although the results obtained are by no means conclusive they indicate several possibilities which may lead to further investigation. Tadpoles of two ages were used, measuring during the period of experimentation from 8 to 10, and 14 to 16 mm. in length. The rates of locomotion were determined by the time needed to swim around a circular track .7 cm. wide and 25 cm. in circumference. This method was suggested by the habit frequently observed in normal tadpoles of swimming around the sides of the bowls in which they are kept.

Very young tadpoles seemed unaffected by a solution of adrenin crystals 1 : 10,000, although older ones showed some response. Solutions of 1 : 1,000 strength were therefore used, and a slight decrease in rate was obtained. As with *Planaria*, this slowing was again due to a greatly increased excitability which made sustained action impossible. After exposure to the extract the tadpoles reacted spasmodically to mechanical stimulation, swimming about in a highly excited state for a short time; but they soon become exhausted and unable to react until after a period of rest. This state of excitability followed by exhaustion was more pronounced in the larger than in the smaller tadpoles, probably indicating an increased sensitivity.

Adrenalin chloride of the same strength had a very powerful effect, producing absolute paralysis of movement in less than a minute. If the animals were removed at once, however, and allowed to recover in water for an hour, the same excitability and lack of endurance was noted as with the solution of adrenin crystals. This seems to bear out the suggestion made at the beginning that it is the chloretone in the preparation which causes the immediate stupefying effect. The same paralysis, moreover, is produced by a solution of chloretone of strength equal to that contained in the adrenalin chloride solution.

With a less concentrated solution of adrenalin chloride, 1 : 10,000, the same effect was produced. At the end of an hour the animals appeared dead; but after another hour in water, the paralyzing effect wore off and they became very active, but unable to keep up any sustained swimming movements. Kept in water for several days, however, the animals, with one exception, completely recovered, and traveled once more at their normal rate. Adrenin, therefore, seems to have an effect upon larval vertebrate muscle as well as upon the simpler muscle of planarians.

This work was carried out under the direction of Dr. Florence Peebles, to whom I wish now to express my sincere appreciation.

Summary.

1. There is a decrease in the rate of locomotion of planarians after treatment with solutions of adrenin, followed by a return to the normal rate when the animals are again placed in water.

2. In those individuals which have not been subjected to the influence of the extract more than an hour, this decrease in the rate of locomotion seems to be due rather to an increased excitability and lack of coördinated movement than to a slowing of the rate of muscular contraction.

3. Planarians exposed to the action of adrenin for a longer period do not give evidence of extreme excitability, but seem to show a real decline in activity, which may be a secondary effect following the initial excitation.

4. Those planarians whose initial rate indicates a state of depression travel more rapidly after treatment with adrenin. This would seem to confirm the suggestion that the effect of this extract upon planarian muscle is predominantly excitatory.

5. Although no definite statement can be made concerning the effects of adrenal extract upon the muscular contraction of toad larvæ, the indications are that they are similar to the effects produced upon the muscular activity of planarians, and that this effect increases with the age of the individuals.

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EYE FACET NUMBER AS INFLUENCED BY TEMPERATURE IN THE BAR-EYED MUTANT OF DROSOPHILA MELANOGASTER (AMPELOPHILA).[1]

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UNIVERSITY OF ILLINOIS.

The effect of temperature upon facet number in the red bar-eyed *Drosophila* was studied in connection with an extended series of experiments on the effect of selection which is in progress in the Zoölogical Laboratory at the University of Illinois.

For the higher temperatures (27°–30°) a bacteriological incubator, varying not more than one degree centigrade was used. The room temperature controlled by a Johnson regulator, recorded by means of a thermograph, and varying not more than 2°, was used for medium temperatures. A cold plate arranged by allowing tap water to flow around a dish in which the *Drosophila* bottles were placed and varying not more than 1° during any one experiment; was used for the low temperature.

The parents in each experiment were taken from mass cultures or stocks, no attempt being made to obtain virgin females for this work. However, the average facet number of the parents of any two sets in an experiment differed but little, any difference occurring being much less than the difference between the averages of the offspring raised at different temperatures. In experiments 14, 15, 17, and 18 the parents were left in the bottles for one day only; in all other experiments they were allowed to remain for nearly the full developmental period in order that a larger number of individuals might be obtained.

That light is not an important factor for facet number was determined by control experiments.

[1] Contribution from the Zoölogical Laboratory of the University of Illinois, no. 141.

The manuscript of Mr. Seyster's paper essentially in its present form was completed in May, 1917, when he entered the U. S. Army Training Camp. At that time it was hoped that he might eventually follow up the work but those plans have not materialized and it seems best to publish the results as they stand. C. Z.

In counting facets, a no. 4 eye-piece and a no. 3 Leitz objective were used. In some cases the facets were counted after the flies had been preserved in alcohol, but mostly the counts were made shortly after the emergence of the flies from the pupa cases. In order to determine the per cent. of error made in counting, a set of ten flies, five males and five females was counted at two different times. The total number of facets in the first count was 1,347, in the second count 1,356, and the per cent. of difference between the two counts was 0.81 per cent. For a larger number of individuals the per cent. of error would probably be less than this; for single individuals it was found to average about 5 per cent. to 7 per cent.

The area of the eye in arbitrary units was found by making an outline camera lucida drawing of the eye, and measuring the enclosed area with a polar planimeter, which was found to give accurate results to 2/100 of a square inch.

DATA.

Experiments 1, 2, 3 (Tables I. and II.).—These experiments were designed originally to test the effect of different amounts of food on facet number. Three bottles, each containing a different

TABLE I.

EXPERIMENTS 1, 2, 3, MALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature.....	(21°–23°)				27°					19–21
Catalog number.....	1	2	3	Total	1	2	3	Total		
Number of individuals.....	11	10	8	29	25	20	21	66		24
Facet averages.....	105	112	106	108	85	86	77	82	26	164
Probable errors.....				±3.0				±1.7		
High variates.....	149	164	126	164	162	178	140	178	14	202
Low variates.....	77	86	84	77	39	45	36	36	41	112

amount of food and thirteen pairs of flies as parents, were placed in a small well-lighted room where the temperature was kept at approximately 27°. After seven days the parents were taken out and their facets counted. It was found that the three bottles differed slightly from one another in facet number but that their

average facet number was decidedly lower than that of the parents. In the male flies this difference was 26, and in the female flies 35. A count of the stock was made at the end of the experiment, and it was found that here the facet number had

TABLE II.

EXPERIMENTS 1, 2, 3. FEMALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature	(21°–23°)				27°					19–21
Catalog number	1	2	3	Total	1	2	3	Total		
Number of individuals	13	12	10	35	30	30	18	78		19
Facet averages	75	89	82	82	47	54	42	47	35	109
Probable errors				±2.2				±1.3		
High variates	100	127	98	127	88	102	86	102	25	146
Low variates	43	52	65	43	31	30	26	26	17	54

increased, the average for the males being 50 higher than that of the parents, and for the females 30. Previous to the time of the experiment, the stock had been kept in a room where the temperature was approximately 21°–23°, while, during the time of the experiment, it was kept at 19°–21°.

Experiment 4 (Tables III., IV.).—Experiments 1, 2 and 3

TABLE III.

EXPERIMENT 4. MALES.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals	10	13	12	35	80	29	17		
Facet averages	135	135	122	131	80	153	121.	32	41
Probable errors				±3.4	±2.6				
High variates	192	174	180	192	161	202	186	16	25
Low variates	101	77	82	77	41	120	87	33	46

indicated that temperature is an important factor in facet variation. To test this more fully one set of *Drosophila* was placed in an incubator, where the temperature was 28.5°, another was kept at room temperature, 22°, and a third set was placed on a cold plate at 17.5°. The offspring were then allowed to

develop at these temperatures. In facet number, those which developed at room temperature gave an average of 121 for the males and 88 for the females, and differed but little from the parents; those which developed at 17.5° averaged 153 for the males and 149 for the females; and those which developed at 28.5° averaged 80 for the males and 45 for the females. In the above experiment, the electric current of the incubator was turned off for one and one half days so that the developing flies of the warm set were at room temperature for that length of time. In

TABLE IV.

EXPERIMENT 4. FEMALES.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals	22	21	24	67	63	18	25		
Facet averages	84	75	76	79	45	149	88	61	43
Probable errors				±1.4			±1.0		
High variates	121	109	113	121	83	188	126	62	43
Low variates	43	57	44	43	20	106	62	44	42

the counts, the effect of this may be noted as about one ninth of the flies is much higher than the rest, the developmental period being nine days at a temperature of 28.5°. The temperature of the cold plate was allowed to rise to that of the room twice for about three hours each time, so that the results are somewhat less striking in this experiment than in some others as regards the difference between high and low facet numbers.

TABLE V.

EXPERIMENT 4. 1. MALES.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17.0°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	10	13	23	19	5		
Facet averages	89	72	79	66	135	23	63
High variates	140	120	140	86	148	54	28
Low variates	60	43	43	44	116	16	73

Experiments 4.1 and 4.2 (Tables V., VI., VII., VIII.).—Part of the offspring of experiment 4 which developed at different temperatures, was used as parents in similar experiments, only here flies developing at the room temperature were not used.

TABLE VI.

EXPERIMENT 4. 1. FEMALES.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	13	17	30	21	4		
Facet averages	54	46	49	58	115	4	69
High variates	83	76	83	94	122	11	46
Low variates	47	31	31	40	106	7	75

TABLE VII.

EXPERIMENT 4. 2. MALES.

	Parents.			Offspring.		Differences Between Parents and Offspring.	
Temperature	17.5°			28.5°	17.0°	17.5°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	158	151	155	94	185	64	34
High variates	202	172	202	138	228	64	26
Low variates	128	131	128	50	136	78	5

TABLE VIII.

EXPERIMENT 4. 2. FEMALES.

	Parents.			Offspring.		Differences Between Parents and Offspring	
Temperature	17.5°			28.5°	17.0°	17.0°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	142	136	139	55	99	87	37
High variates	163	165	165	74	121	89	44
Low variates	108	106	106	28	65	80	41

The results obtained from all four sets are analogous to those obtained from experiment four. The results from the above experiments indicate that the temperature at which the parents

[1] Development at room temperature for one and one half days.

develop has little, if any, effect upon the facet number of the offspring.

Experiment 5 (Tables IX., X.).—This experiment is the most conclusive as a high temperature of 29° and a low temperature

TABLE IX.

EXPERIMENT 5. MALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature	22°			29°	17.5°	
Catalog number	5.1	5.2	Total	5.1	5.2	
Number of individuals	10	10	20	86	64	
Facet averages	129	127	128	56	167	111
Probable errors			±5.6	±0.9	±3.0	
High variates	200	211	211	98	226	128
Low variates	90	80	80	34	74	40
Coefficient of variation			25	24	22	

of 17.5° were maintained throughout. The male flies developing at the low temperature showed an average facet number three times as great as that of those developing at the higher temperature, the corresponding ratio for the female flies being 4.7. The average facet number of the male flies developing at 29° was 56, of those developing at 17.5°, 167; the average facet

TABLE X.

EXPERIMENT 5. FEMALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature	22°			29°	17.5°	17.5°–29°
Catalog number	5.1	5.2	Total	5.1	5.2	
Number of individuals	12	12	24	71	75	
Facet averages	97	87	92	27	128	101
Probable errors			±3.0	±0.5	±1.5	
High variates	138	144	144	43	188	145
Low variates	34	53	34	16	83	67
Coefficient of variation			25	21	20	

number of the females developing at 29° was 27, of those developing at 17.5°, 128. The parents of the above sets, developing at 22° showed little difference in the average facet number. In the males this difference was 2 facets, in the females it was 10. In both males and females the parents which had the higher facet average gave offspring with the lower average, these being

the ones that developed at 29°. The extreme variates (high, low) of the parents of the two sets are slightly greater in the case of the set which developed at the lower temperature, but as the greater difference here is only eleven facets, and as the difference between the variates of the two sets of offspring is as great as 145, it is certain that selection can play no great rôle in determining the facet difference of the offspring here noted.

Experiment 5.1 (Tables XI., XII.).—Parents of this experiment are the offspring of set 5.1 in experiment 5. In experiment 5.1, one set of offspring developed at 29° while another developed at 17°. The male flies developing at 29° gave an average which was 5 facets less than their parents which developed at the same temperature while the male offspring developing at 17° had a facet average which was 127 greater than that of the parents. The facet average of the female offspring was 11 greater for those developing at 29° and 120 greater for those developing at 17°, than that of the parents which developed at 29°.

TABLE XI.

EXPERIMENT 5. 1. MALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		7	21		
Facet averages	56	51	183	5	127
High variates	98	61	303	37	205
Low variates	34	38	127	4	93

TABLE XII.

EXPERIMENT 5. 1. FEMALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		16	26		
Facet averages	27	38	147	11	120
High variates	43	55	235	12	192
Low variates	16	27	111	11	95

A part of the offspring from set 5.11 was continued for three more generations at 29° (Table XIII.). The fifth generation

gave an average of 63 for the males and 35 for the females. The males were lower and the females higher than the first generation (Experiment 5) which developed at 29°.

TABLE XIII.

THREE MORE GENERATIONS AT 29°—A CONTINUATION OF THE 5.11 LINE.

	Males.			Females.		
Generation number	3	4	5	3	4	5
Facet averages	42	26	63	35	44	35
Number of individuals	17	7	55	9	25	52
High variates	58	86	130	46	56	49
Low variates	32	43	31	28	25	20

Experiments 14, 15, 16, 17, 18 (Tables XIV., XV., XVI., XVII., XVIII.).—In experiments 14, 15 and 17, the effect of temperature on the white bar-eye *Drosophila* was determined,

TABLE XIV.

PARENTS. EXPERIMENTS 14, 15, 17. DEVELOPED AT 27° C.

Males.

Catalog Number	14.1 15.3 17.2	14.2 15.4 17.3	14.3 15.5 17.4	14.4 15.6 17.5	14.5 15.1 17.6	14.6 15.2 17.1
Number of individuals	16	14	15	6	12	15
Facet averages	117	115	106	120	119	108
Probable errors ±	4.6	6.0	3.0	6.9	5.9	4.8
High variates	166	206	136	126	161	184
Low variates	56	60	72	82	82	75

Females.

Number of individuals	24	22	19	18	18	23
Facet averages	73	69	77	74	75	75
Probable errors ±	2.6	2.2	2.2	2.7	2.2	2.2
High variates	107	103	116	110	102	102
Low variates	44	44	57	47	38	40

and the results are fully concurrent with the results obtained from the work on the red-eye. Experiment 18 gives additional data for the red bar eye.

These four experiments were made in order to determine the period of development when temperature is most effective. To determine this, five bottles with parents were placed at 29° and the developing flies in the different bottles were allowed to pass different fractions of their developmental period here. They were then removed to the cold plate at 15°, or to the room at

22°, and allowed to complete their development at these temperatures. One bottle was kept on the cold plate throughout the period of development, and another was kept at 29°. Removals

TABLE XV.

EXPERIMENT 14. OFFSPRING. PART OF DEVELOPMENT 29°—REST AT 15°.

Males.

Catalog Number.	14.1.	14.2.	14.3.	14.4.	14.5.	14.6.
Number of days at 29°	0	½	2	4½	6½	all
Developmental period in days	33	31	28	21½	10	8
Number of individuals	6	9	13	6	37	22
Facet averages	282	272	352	57	64	76
Probable errors ±	13	7.3	9	2	2.4	1.7
High variates	366	325	414	65	130	161
Low variates	240	217	280	52	45	39
Females.						
Number of individuals	10	21	18	10	25	21
Facet averages	215	266[1]	231	56	56	59
Probable errors ±	11	16	5	2.4	1.4	1.1
High variates	288	650[1]	281	84	86	80
Low variates	147	190	171	43	39	48

from 29° to a lower temperature were made as follows: Number 2, after one half day; number 3, when the larvæ were about one third grown (2–2½ days); number 4, just before pupation

TABLE XVI.

EXPERIMENT 15. OFFSPRING. PART OF DEVELOPMENT AT 29°. REST AT 15°.

Males.

Catalog Number	15.1	15.2	15.3	15.4	15.5	15.6
Number of days at 29°	0	½	2½	4½	9	all
Developmental period days				21	11½	9
Number of individuals	4	17	4	2	21	19
Facet averages	304	271	293	76	63	60
High variates	324	402	326	88	88	98
Low variates	290	161	248	65	40	42
Females.						
Number of individuals	2	18	8	2	27	32
Facet averages	172	188	233	41	41	43
High variates	180	233	289	47	66	63
Low variates	164	130	178	36	33	27

had started; number 5, after most of the larvæ had transformed into pupæ. Number 6 was kept at 29°. In experiment 18, removals from room temperature (22°) to 29° were made, and

[1] Average includes female with 650 facets. She may be heterozygous for bar.

instead of using white bar-eyed flies, the red bar-eyed were used. As the parents were kept in the bottles for one day, the length of time the developing flies were allowed to remain at the initial

TABLE XVII.

EXPERIMENT 17. PART OF DEVELOPMENT AT 29°. REST AT 22°.

Males.

Catalog Number	17.1.	17.2.	17.3.	17.4.	17.5.	17.6.
Number of days at 29°	0	½	2½	4¼	7½	all
Developmental period days	12	12	12	11	8½	8½
Number of individuals	31	6	5	45	25	25
Facet averages	131	170	127	102	65	69
Probable errors ±	2.9	7.4	8.1	2.4	3.6	2.3
High variates	171	198	180	150	144	129
Low variates	99	125	96	36	32	42

Females.

Number of individuals	32	8	4	50	30	29
Facet averages	114	130	101	66	39	44
Probable errors ±	2.4	3.0	5.1	2.3	1.0	1.0
High variates	172	160	117	132	62	61
Low variates	80	112	80	32	25	31

temperature was reckoned from the middle of that day and not from the beginning of the experiment, in order to give the mean time of development at that temperature for all the eggs laid during that day.

TABLE XVIII.

EXPERIMENT 18. PART OF DEVELOPMENT AT 22°. REST AT 29°. RED BAR-EYE.

Males.

Catalog Number.	18.1.	18.2.	18.3.	18.4.	18.5.
Number of days at 22°	0	2½	6½	9½	all
Developmental period days	8¾	9¾	11	11¾	12½
Number of individuals	9	12	11	14	8
Facet averages	89	98	146	175	189
High variates	167	166	164	210	291
Low variates	49	64	65	128	124

Females.

Number of individuals	4	11	13	19	12
Facet averages	40	45	104	150	130
High variates	47	68	172	183	213
Low variates	35	35	52	120	101

In experiments 14 and 15 the effect of temperature is to be noted early in the larval period; in 17 and 18, which were changed from 29° to 22° and from 22° to 29° respectively, the effect of

temperature is to be noted throughout the larval life. In no case was there any significant effect upon the facet number of the flies after the pupæ had been formed. The parent flies of the six different sets differed but little in facet number. In experiments 14, 15 and 17 each set of parents was used in each experiment. For instance, the parents of 14.1, 15.3 and 17.2 were identical. In experiment 18 the same parents were used throughout, being changed from one bottle to another.

Facet Change per Degree Change in Temperature.—With one exception, the number of facets per degree change in temperature varies from 5.2 to 8.9 and is fairly constant. The following is a list of facets per degree change, found by dividing the difference in facet averages by the difference in degrees centigrade:

Experiment.	Temperatures.	Facet Average Low Temperatures.	Facet Average High Temperatures.	Facet Differences.	Facet Difference. / Temp. Difference.
1, 2, 3, males.....	22° −27°	108	82	26	− 5.2
1, 2, 3, females.....	22° −27°	82	47	35	− 7.0
4 males.....	17.5°–22°	153	121	32	− 7.1
4 females.....	17.5°–22°	149	88	61	−13.5
4 males.....	22° −28.5°	121	80	41	− 6.3
4 females.....	22° −28.5°	88	45	43	− 6.6
5 males.....	17.5°–29°	167	56	111	− 8.9
5 females.....	17.5°–29°	128	27	101	− 8.0

The relation between the facet number and a ten degree difference in temperature is also very interesting. Is the number of facets increased two to three times per ten degrees decrease? The following table gives the results, n_t being the number of facets at the lower temperature and n_{t+10} at the higher temperature:

Experiment.	♀ $\frac{n_t}{n_{t+10}}$.	♂ $\frac{n_t}{n_{t+10}}$.
1, 2, 3	2.6	3.5
4	1.7	3.0
5	2.5	3.9
5.1	3.0	3.2
Average,	2.6	3.5

Size of Individual Facets (Plate I.).—The relation of the area of the eye to facet number was found to be constant for flies with an intermediate number of facets. This constant, for 43 individuals, was found by dividing the facet number by the area of

the camera lucida drawing in 1/100 sq. in. In flies with a very small number of facets the relation does not hold; in very large eyes, the curved surface makes it difficult to obtain accurate areas. Leaving out of consideration these extremes, the change in facet number is accompanied by a corresponding change in the size of the eye, and there is no change in the size of facets.

Discussion.

Observations upon the red and white bar-eye show that temperature is an important factor in determining facet number of the flies taken at random from a general population. Flies raised at a higher temperature, however, do not have a higher number of facets, but on the contrary, the higher the temperature of development, the lower the facet number. An explanation which may be offered presupposes the existence of a chemical factor or determiner which acts as an inhibitor of facet formation and that, at a higher temperature, the speed of reaction is much greater than at a lower. Then, according to Van't Hoff's law, with an increase of 10° C. the speed of the reaction should be from two to three times as great and the number of facets one half to one third times as large, and vice versa. An examination of the data shows a considerable degree of approximation to this condition. In the males with a 10° decrease the facet average is 2.6 times as great; in the females 3.5 times. Whether or not the high value, 3.5, in the females is due to the fact that bar-eye is sex linked and the female receives two chromosomes containing this factor, while the male receives but one, is a matter for speculation.

Summary.

1. Temperature is an important factor in the determination of facet number in the bar-eye of *Drosophila*.

2. A lower developmental temperature results in a higher facet number, and conversely a higher temperature results in a lower facet number.

3. With each 10° drop in temperature between 29° and 15°, the facet number is increased on the average 2.6 times in the males and 3.5 times in the females.

4. The increase in facet number except at the extremes is

directly proportional to the increase in the area of the eye, and facet-size is therefore a constant within these limits.

5. Light, and amount of food, as they occur in these experiments are not important factors in the determination of facet number.

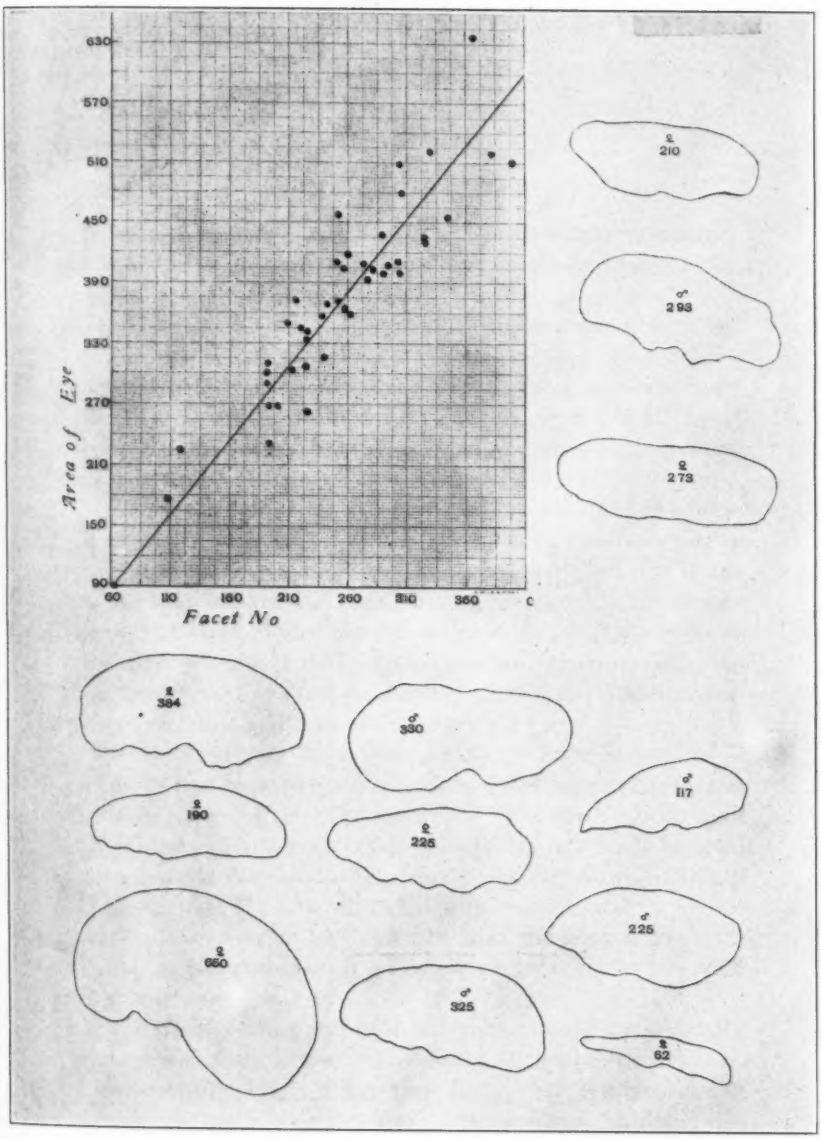
6. Temperature is effective only during the larval period.

ACKNOWLEDGMENT.

This work was done under the direction of Dr. Charles Zeleny and I wish to express my thanks to him for his interest in the problem and for his many suggestions while it was in progress.

Explanation of Plate I.

Relation of facet number to area of eye in white bar-eye. Distribution of forty-three cases around average represented by line. Camera lucida drawings of eyes, with facet number.


630
570
510
450
390
330
270
210
150
90
60
110
160
210
260
310
360
Area of Eye
Facet No
♀ 210
♂ 293
♀ 273
♀ 384
♂ 330
♂ 117
♀ 190
♀ 225
♂ 225
♀ 650
♂ 325
♀ 62

E. W. SEYSTER.

CIRCULATION OF THE CŒLOMIC FLUID IN A NEMATODE.

FREDERIC H. KRECKER.

The nematodes are not known to have a special circulatory system and it has been rather generally considered that they do not possess a circulating medium.

In some nematodes of the genus *Camallanus* taken from the rectum of a sunfish (*Apomotis cyanellus*) I noticed a flowing movement of the cœlomic fluid. It was blood red and could therefore be easily distinguished. Waves of the fluid passed from the anterior to the posterior end and then in the reverse direction.

The reversal of the circulation calls to mind the condition in ascidians. In them it will be remembered, there is a clearly defined circulatory system and the reversal of blood flow is due to the heart forcing the blood first in one direction for twenty or thirty beats and then, after a pause, sending it in the opposite direction. The only similarity in these two instances lies in the fact that in both of them the circulating medium is impelled first in one direction and then in the other.

Before describing fully what occurs in these nematodes it might be best to point out briefly such of their structural features as have a bearing on the phenomena. The specimens at hand are all females from 20 to 25 mm. long. As commonly happens among nematodes the uterus extends practically the entire length of the cœlom. It is so distended with embryos that in optical section it appears to be in contact with the body wall. As a matter of fact there is just enough space between the two for a thin layer of cœlomic fluid.

The circulation of the fluid is brought about by peristaltic contraction of the uterus. The ability of these constrictions to force the fluid along is due to there being more of it than can be accommodated in the narrow peri-uterine space. A sharp constriction of the uterus forms a comparatively deep depression

which becomes filled with cœlomic fluid. This constriction moves as a wave lengthwise of the uterus and carries fluid along because the latter cannot easily escape.

The description of the entire process is as follows, beginning, for the sake of convenience, when the fluid leaves the posterior end. The peri-uterine space of this end is at this time filled with cœlomic fluid for approximately one-sixth of the worm's entire length. A peristaltic wave arises at the posterior end of the uterus and moves anteriorly. This is followed by another, then a third and sometimes a fourth. Each wave carries with it some of the fluid, the first taking the largest or primary wave of fluid, the third or the fourth, if there is a fourth, taking the least. The three waves all leave within seven seconds at slightly over two second intervals. After the last wave has left, only a thin film of fluid remains in the peri-uterine space. At about the time when the first of the peristaltic waves starts from the posterior end of the uterus, posterior to anterior waves of peri stalsis are to be seen at its anterior end, each of them pushing along a little fluid. It was rather difficult to watch both ends of the worm at the same time and so my observations on what were practically synchronous occurrences may be subject to slight error. There are usually three of these peristaltic waves at the anterior end and then, with the fourth, comes the primary wave of fluid from the posterior. This wave arrives at approximately the time when the third wave is leaving the posterior end. The circulation is therefore a rather sluggish process since the time between the first and the third waves is seven seconds and the entire length of the worm is only twenty-five millimeters.

As soon as approximately one sixth of the cœlom at the anterior end has been filled with fluid, anterior to posterior peristalsis begins at this end and continues in the manner just described for the posterior end; a primary wave of fluid leaves, followed by a second and a third and sometimes a fourth, the first wave being the largest and each succeeding one smaller. There is this difference, however, between the processes at the two ends; when a peristaltic wave arises at the anterior end of the uterus it pushes cœlomic fluid both anteriorly and posteriorly instead of sending it all posteriorly. This movement in opposite

directions occurs because the uterus does not completely fill the anterior end of the cœlom and consequently when it constricts it allows some fluid to move anteriorly. As a result, there is always a certain amount of fluid left at this end even after the last antero-posterior peristaltic wave has passed on. Another point of difference in the processes at the two ends is the fact that at the posterior end there is an instant's pause after the arrival of the fluid before it is sent back, while at the anterior end there is no pause, the peristalsis being immediately reversed. In view of this pause the posterior end can be considered as marking the beginning and the ending of the circulatory cycle. Seven cycles of the primary wave occur in two minutes. This rate was maintained by each of two worms in which the process was timed.

Frequently all of the secondary waves moving in a given direction have not reached the end toward which they are travelling before a primary wave starts toward them from that end. When this occurs the secondary wave keeps on moving until it meets the primary wave which then absorbs it and continues onward. When, as in the case of the posterior end, there is a momentary pause in the peristalsis of the uterus, the fluid does not necessarily come to rest throughout the entire cœlom, but secondary waves which may be moving keep on their course until they have reached the posterior end or until the primary wave from that end has met them.

Just why these secondary waves continue to move in this manner my observations did not make quite clear. The condition might be brought about by peristalsis from one end continuing until it met and was overcome by that originating at the opposite end. I am not prepared to say that this is the case, especially since, postero-anterior peristalsis begins at the anterior end almost synchronously with that at the posterior end. Possibly the impetus received from the peristalsis is sufficient to keep the secondary waves moving for a time even after peristaltic action has ceased.

As a wave moves along, a certain amount of the fluid composing it trails behind and comes to rest. This slipping back occurs because the uterus does not completely fill the cœlom.

The size of a given wave is maintained by carrying along the fluid in its path. As a result of the trailing fluid there is always some of it all along the cœlom. This residuum explains the arrival of small quantities of fluid at a given end with the peristalsis which precedes the arrival of the primary wave. The free play between uterus and body wall explains also why all of the fluid is not carried away from an end with the first peristaltic wave but is instead taken off in lessening quantities by several waves.

What relation the movement of the cœlomic fluid has to the well-being of the adult worm or its young depends upon the general function and composition of the fluid. Very little definite information has been published regarding this matter for the nematodes in general, and nothing, so far as I am aware, on the fluid of the genus *Camallanus*. It has been suggested that one of the functions served, in some cases at least, is to act as a medium for oxygen. If this be true the circulation in the present instance can be looked upon as of value in effecting a thorough distribution of the available oxygen.

There is some reason for believing that the circulation is of particular benefit to the young. Pointing to this conclusion is the fact that the peristaltic waves very effectively stir up the young and constantly bring different individuals into contact with the walls of the uterus and therefore nearer the cœlomic fluid.

From present knowledge it is rather hard to conceive what other purposes than those suggested would be served by such rhythmic constrictions of the uterus. Especially is it hard to see why there is a continual stirring up of the embryos bringing successively different individuals into contact with the walls of the uterus as is done in this case if it were not for some purpose connected with the surrounding medium. There is, however, a rather extensively held opinion that the cuticle of nematodes is impervious to all but the strongest fluids and even in some cases to strong formalin. If this were true the cuticle could possibly prevent absorption of oxygen. The view just mentioned is based on such examples as the vinegar eel, and on some instances in which adults of other species or their contained embryos have resisted preserving or fixing reagents for a considerable length

of time. It is doubtful whether careful or extensive work would prove this imperviousness to be as general or as great as it is sometimes thought to be.

The fact that the cœlomic fluid is red points suggestively to an oxydizing function. In other nematodes having red cœlomic fluid the color is said to be due to the presence of hæmoglobin and this is therefore probably the condition in these worms. Since their host is an aquatic animal and since they live rather near the anus, it would be a comparatively easy matter for a certain amount of oxygenated water to come in contact with them. This being true, hæmoglobin in the cœlomic fluid could take up any available oxygen.

In dissolving any doubt regarding the function of circulation in these worms it would be materially helpful to learn how extensive the process may be among nematodes in general and under what conditions it occurs. There is so little color differentiation in the fluid of most nematodes that possibly circulation may have been overlooked in species where it does actually take place. In the present case the redness of the fluid in contrast with the light background of the tissues made movements of the fluid noticeable and easy to follow. If the process is primarily an oxygenating one it might be found more frequently among species internally parasitic or otherwise living where thorough distribution of a small supply of oxygen would be a matter of importance.

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THE INFLUENCE OF CERTAIN DUCTLESS GLAND SUBSTANCES ON THE GROWTH OF PLANT TISSUES.[1]

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That thyroid gland constituents and secretions contain substances which effect clear, and often far-reaching, influences on the metabolism and action of animal cells has long been recognized, both from observations of natural wild stock, and from experimental data. As a normally produced hormone, or as a substance introduced artificially, thyroid substances alter the norm of the metabolism of an adult tissue; in no less, but rather in more pronounced ways, thyroid tissue fed to growing embryos modifies their growth and differentiation.

Proof of the above has been furnished by numerous workers for vertebrates, especially for representatives of the mammalia and amphibia; and for insects and insect larvæ, as Northrop and Kunkel, respectively, have demonstrated. Protozoa also show a marked modification of their metabolic processes when they feed upon or absorb thyroid gland products, as indicated by the work of Nowikoff, Shumway, Budington and Harvey, and more recently by Chambers.

After noting that living substances in organisms of such widely different constitution and phylogenetic position seem distinctly susceptible to this hormonic material, the question arises,—Is thyroid substance a compound with special potencies over animal metabolism, or is all protoplasm amenable to its influence? Assuming a single origin of living material, has the wide divergence of the animal and plant kingdoms produced in the latter a type of protoplasm which may be immune to this glandular product, so foreign to plants in its place of synthesis?

This paper reports the morphological effects of thyroid constituents on the growth of the root-tips of the onion, *Allium*. To avoid seasonal eccentricities, the experiments were repeated

[1] From the Department of Zoölogy, Oberlin College.

three different years; and in this connection I am very glad to acknowledge much assistance from three senior students, Miss Helen F. Harvey, Miss Harriet M. Heeman and Miss Gladys Newman.

Methods and Results.

The experiments were carried out in mid-winter and early spring when the dormant period of onion bulbs is naturally terminating. Material was secured from the open market, from different dealers for successive trials; sound bulbs of medium size, suited to nicely cover the open tops of Naples staining jars of 45 mm. diameter, and 120 c.c. capacity, were selected; care was taken to discard any which already showed root growth.

The nutrient fluid used in the jars was Pfeffer's solution, made up according to the formula given in Duggar's "Plant Physiology." [1] Each experiment consisted of five or more groups of bulbs; to the nutrient fluid in jars supporting them was added desiccated thyroid gland (in tablet form put up by Parke, Davis and Co.) in the following amounts: Group I., 1 grain; Group II., 0.75 grain; Group III., 0.5 grain; Group IV., 0.25 grain; Group V. was the control, which grew on the nutrient solution alone. The solutions were stirred from time to time to secure homogeneity. Some experiments were carried out in a greenhouse affording approximately uniform temperature and humidity; but results obtained under these conditions did not differ from those conducted in the common laboratory environment.

A further detail may also be mentioned here; it was natural that solutions such as were used, containing organic substances, would gradually become infected with bacteria, no matter how sterile the ingredients at first. This feature of the procedure

[1] Pfeffer's solution as used:

Calcium nitrate	4 grams.
Potassium nitrate	1 "
Magnesium sulfate	1 "
Potassium dihydrogen sulfate	1 "
Potassium chloride	0.5 "
Iron chloride	trace.
Water dist.	5 liters.

would be of more importance in case the solutions were employed for prolonged periods; but in this work the bacterial factor never seemed to interfere with entirely healthy root growth. Indeed, Curtis has shown that, in some instances at least, a heavy infection of bacteria and fungus filaments about root sprouts on cuttings seems to be favorable to their better growth. His experiments covered many weeks, often months; deleterious effects due to carbon dioxide production in the solution may follow such infection after extended periods; but the factor is negligible in experiments limited to ten-day or two-week periods.

The following photograph is essentially self-explanatory and may be taken as typical of many. Roots grew somewhat in

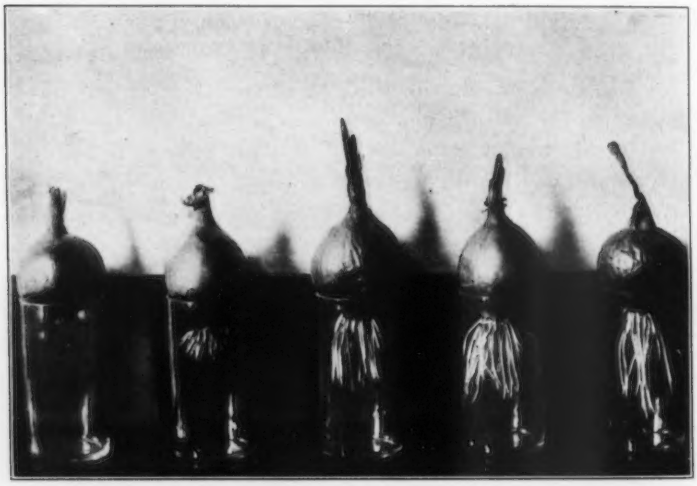

FIG. 1. Onion bulbs growing in Pfeffer's nutrient solution, in which is dissolved desiccated thyroid gland material, in parts of a grain indicated.

solutions of each different strength here mentioned; the very slight growth in the left hand bulb here figured would have continued to greater length after a time, but the proportionate lengths would have remained much as when the picture was taken. The apparent influence of the thyroid material is to retard growth rate.

Discussion.

The almost constant effect of abnormal amounts of thyroid tissues or extracts in the food or environment (or both) of developing animals has been to accelerate differentiation in premature ways. So too, one may interpret increased fission rate in Protozoa as indicating a similar effect, fission being a procedure characteristic of their attainment of adult physiological conditions. Such precocious metamorphosis of amphibian and insect larvæ is naturally coincident with small size.

The difficulties in estimating the effect of substances on plant tissues in terms of animal response and metabolism are obvious, and one should perhaps heistate to make any comparisons. However, all protoplasm obtains its raw materials through its permeable cell walls; and one may also go further and say that the root tips of bulbs, as they begin to grow, represent a kind of embryonic tissue with a minimum of specialization. In brief, the effect of thyroid substances on onion root-tips is to retard, or partially inhibit, size-growth; and this seems precisely the effect on embryonic animal tissue. In the latter, a hastening of physiologic and morphologic differentiation is also present; if the same be true in these root-tips, it naturally cannot be judged from external appearances, though one cannot deny that premature differentiation may be present. It is hoped to report on this point in a later communication.

Since the work of Marine and Lenhart, Morse, Swingle and others has made certain the earlier assumption that iodine is the most active principle in thyroid stuffs, a series of experiments in which only iodine was added to the nutrient solution was carried out. To make more significant and exact the comparison between such an experiment and those in which dessicated gland was used, KI was added in amounts based upon the accepted estimate that 0.01 c.c. of a saturated solution of KI furnishes the amount of iodine present in one grain of thyroid. Onion root-tips sprouting in media with this iodine content showed no growth-rate which was specifically different from that of controls growing in nutrient solution alone.

Further sets of experiments were carried out with pituitary gland tablets, using strengths of 2, 1.5, 1 and 0.5 grains in 120 c.c.

of the nutrient fluid; supra-renal gland tablets were also used, in strengths of 1, 0.75, 0.5 and 0.25 grains in 120 c.c. of nutrient fluid; but there was no uniform effect of these substances, the appearance being that these substances so used have no influence at all on this particular plant tissue.

SUMMARY.

1. Growth of root-tips of *Allium* is retarded by the presence in their fluid nutrient environment of thyroid gland material. Retardation is approximately in direct proportion to the amount of thyroid substance present.

2. The presence of thyroid materials in the nutrient fluid in which *Allium* is sprouting does not modify the growth of the early leaves.

3. Iodine, used as KI, in amounts equivalent to that in thyroid substances provoking marked modifications of growth, has no appreciable effect on growing root-tips.

4. Pituitary substances up to two grains of the desiccated gland, and supra-renal substances up to one grain of the desiccated gland in 120 c.c. of nutritive solution have no effect on the growing root-tips of *Allium*.

5. While no general conclusion can be based on experiments limited to a single form, the indication is that thyroid constituents may influence the rôle of protoplasmic action in cells other than those of animal tissues.

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STUDIES ON THE ROTIFER ASPLANCHNIA EBBESBORNII, WITH SPECIAL REFERENCE TO THE MALE.

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INTRODUCTION.

The rotifers were collected in small rain pools, placed in aquaria with tap water and kept in the laboratory at room temperature, free from direct sunlight. The age of the cultures, when the present work was begun, varied from four months to two years. The rotifers appeared in the different cultures at regular intervals of about six to eight weeks.

The life cycle of *Asplanchnia* is as follows: females hatch from the resting or fertilized eggs. These females (first generation) produce parthenogenetically twenty to thirty offspring, which are likewise females. The latter reproduce parthenogenetically. All of the offspring of one parent, being either all females or males. Thus the females of the second generation may be called male producers or female producers. The life cycle of *Hydatina senta* as described by Shull, in most respects corresponds to the above description.

When the males become sexually mature, impregnation of the male-producing females occurs, and the impregnated females produce resting eggs instead of males or both resting eggs and males. The resting egg stage terminates the active period of each cycle and carries the rotifers to the beginning of the active period of the next following cycle. The active free-swimming period of each cycle continues from two to three weeks. The inactive or resting egg stage of each cycle varies from five to eight weeks. If impregnation of the male producers is prevented, no resting eggs are formed and the females continue to form males parthenogenetically.

If we consider the females that actually produce males the sum total of all male producing females formed, the percentage

of the male producers would be rather low and remain constant regardless of external conditions. But if we include the resting egg producers (potentially male producers) in the sum total of the male producers the percentage under favorable conditions often reaches 90 to 95 per cent.

In this particular rotifer there are two kinds of resting eggs, which require fertilization before development begins. The one (single shelled) develops within the uterus of the parent with the same rapidity as the parthenogenetic forms and hatches out immediately when deposited. The other (double shelled) with the exception of a few early cleavage stages, develops after deposition.

No attempt was made to regulate the food conditions of the different cultures, except the adding of tap water at different times to counteract evaporation. The amount of food available for the rotifers varied greatly during the active periods of the different life cycles. Some of the cultures were almost entirely free from food of any kind, while other cultures were rich in euglenæ and unicellular plants or animals. It was found that the number of male- and female-producing females varied according to the amount and kind of food present. Scarcity of food favored the production of female-producing females. Culture rich in euglenæ and unicellular plants, favored a high production of male-producing females.

Experiments in mating and sex determination have been carried on extensively by Shull, Whitney and others but will not be considered here, since they do not come directly within the domain of the present investigation. However, some very suggestive features on sex determination present themselves in the study of the above rotifer. It is true that certain food conditions favor a high production of male-producing females, but immediately after impregnation, as stated above, these same male-producing females begin the production of resting eggs, or both male and resting eggs, which may alternate in the same female. Again it is not an unusual thing to find within the oviduct of the male-producing female embryo before birth, either mature male eggs or male eggs that have begun to develop, and if impregnation of these male producers occurs after birth, resting eggs

instead are produced later. The question of sex determination from the standpoint of external conditions is rather an intricate problem to attempt to solve, since the same male-producing females are capable of producing simultaneously both males and female-producing females (from thin-shelled resting eggs) under the same food conditions.

Comparative Study of Male and Female.

The males with few exceptions as found in the different groups of rotifers are smaller than the females. The cleavage and early development in both sexes are quite similar. Previous investigators have based their results almost entirely on the study and development of the females and have not made a careful study of the degree of development and degeneration as it occurs in the male, more especially when compared with the conditions found in the female.

The development of the parthenogenetic female is represented in the series of diagram A, 1–15, and the parthenogenetic developing male in the series of diagrams B and C, 1–15. In case of the male the series B represents the condition, where degeneration of the male individual is very pronounced. Series C represents the condition of normal development, where only partial degeneration of the male occurs.

A. Formation and Maturation of Ova.

The ovary of female-producing male individuals and resting eggs and of the female-producing males are quite similar (Figs. 1 and 2). It is composed of a group of very small cells (ova), situated on the convex side of the vitellarium, near the point where the oviduct takes its origin. These small cells are uniform in size, and at regular intervals one of them begins its growth and becomes the mature ovum. Occasionally two begin their growth simultaneously. The growth is very rapid and the cytoplasmic granules pass directly from the vitellarium into the growing egg (Figs. 1 and 2). When growth is complete the egg is separated from the ovary and passed into the upper end of the oviduct where maturation occurs. The origin and formation of the male, female and resting eggs are quite similar. The rest-

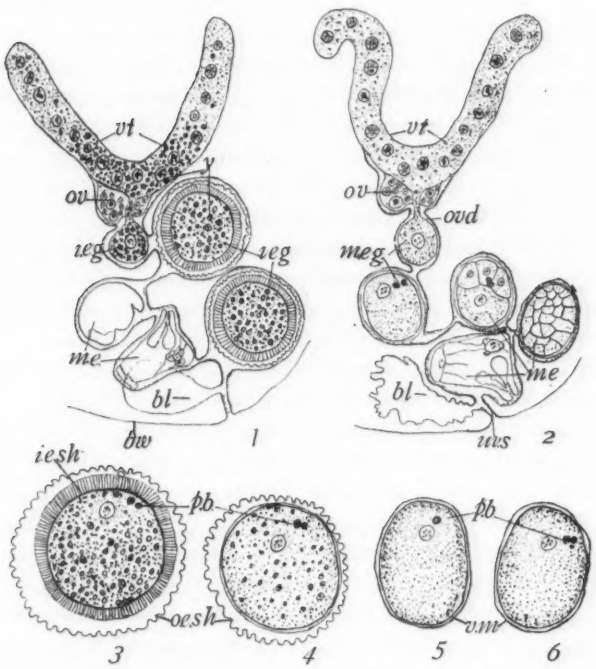

FIG. 1. Represents the reproductive system of a male-producing female, which shows the presence of both male embryos and resting eggs. Where male embryos and resting eggs alternate, the yolk is formed at intervals just preceding the growth of the resting eggs. The figure shows the passage of yolk into the forming resting egg.

FIG. 2. Represents the reproductive system of a male-producing female, which contains male embryos in different stages of development. The vitellarium is free from yolk and shows the passage of cytoplasmic granules into the forming male egg. One of the male embryos shows the condition at time of birth.

FIG. 3. A mature double-shelled resting egg, which is filled with considerable yolk.

FIG. 4. A mature single-shelled resting egg, which contains less yolk than the preceding. Its development is completed within the uterus and hatches immediately after deposition.

FIG. 5. A mature parthenogenetic female egg, which is entirely free from yolk. A single polar body is formed.

FIG. 6. A mature parthenogenetic male egg, which has the same structure as preceding. Two polar bodies are formed. All of the above eggs show the presence of a well-formed vitelline membrane.

ing egg (double shelled) is represented in Fig. 3. The outer shell membrane is external to the vitelline membrane and is formed by a secretion from the uterus. The inner protective membrane is formed from the cytoplasm within the vitelline membrane. Fig. 4 represents the single-shelled resting egg, which contains less yolk than the former. Two polar bodies are formed in either case. The female parthenogenetic egg (Fig. 5) is free from yolk and has a single polar body. The male egg (Fig. 6) with the exception of the two polar bodies is indistinguishable from the female parthenogenetic egg. The cytoplasmic content of the above eggs from the standpoint of quantity is about the same.

Impregnation of the male-producing female brings about a new condition in the formation and growth of the resting egg. The vitellarium instead of remaining transparent as in the formation of the parthenogenetic male and female eggs, becomes filled with numerous spherical yolk bodies, which pass directly from the vitellarium into the growing resting egg (Fig. 1, *r.eg*). These yolk-filled eggs are very dark, and unless fertilization occurs further development stops after maturation. The eggs deteriorate and do not give rise to males as advocated by some of the investigators on rotifers. The growth period of the female, male and resting eggs with the passage of cytoplasmic granules or yolk bodies from the vitellarium into the growing eggs, as well as the maturation stages can be demonstrated under the microscope in the living individual, the entire process requires about one hour. In this particular rotifer there can be no question as to the structural differences in the male and the resting eggs. The male eggs are always transparent, free from yolk and incapable of being fertilized. Whether the male and the resting eggs are the same or not before their growth period begins in the ovary of the male-producing females I am unable to say. There is a possibility of two distinct kinds of ova being present: the one which normally becomes the male egg, the other which requires impregnation before the growth period begins in the formation of the resting egg. The ova that become resting eggs never begin their growth unless impregnation occurs.

B. Cleavage and Gastrulation.

It is not my intention to give a detailed account of the different cleavages. A brief account of the more important stages of development, as they occur in the parthenogenetic female and male will be considered for the purpose of comparison in emphasizing the varying degrees of development and degeneration in these organisms.

The development of *Asplanchnia ebbesbornii* has not been worked out by previous investigators. Complete development (except of the double-shelled resting egg), occurs within the oviduct and uterus of the parent. The animals are extremely transparent so that it is possible to make a study of the various cleavage stages as they occur within the living body. The eggs are extremely plastic with a well-defined vitelline membrane (Figs. 5 and 6). The nuclear activities are distinct in the living cells, and the order, rate and direction of the cleavage spindles can be followed without any difficulty. Preserved material if stained, dehydrated, cleared and mounted in damar immediately after fixation, serves as a fine check in the study of cleavage. The cell lineage of *Asplanchnia Herricki*, from early cleavage to late gastrula, was worked out by Jennings. His entire work was based upon the study of preserved material. The rotifers were fixed in toto, the eggs dissected out and studied under the cover slip. With few exceptions he was unable to verify the presence of a distinct egg membrane. He seldomly found more than a single embryo in the same uterus.

The cell lineage of *Asplanchnia ebbesbornii*, for the most part is a confirmation of Jennings' results. The eggs are somewhat elliptical with the cleavage nucleus nearer the anterior end (A–C, 1). The first cleavage plane passes through the region of the polar body or bodies and divides the egg into two very unequal cells (A–C, 2). The second cleavage occurs at an angle of about forty-five degrees to that of the first, dividing the anterior smaller cell equally and the posterior larger cell unequally (A–C, 3 and 4). The four macromeres show considerable uniformity in the condition of their cytoplasmic structures. The region immediately surrounding the nuclei is more transparent and free from cytoplasmic granules. In the third cleavage the

large cell D (A–C, 4) divides first. The ectomere d^1 is budded off in an upper anterior direction on the median dorsal side and covers the posterior ends of the macromeres C, B and A. The cells C, B and A before division are drawn out in an anterio-posterior direction and divide nearly equal. The ectomeres c^1, b^1 and a^1 are on the same level with their parent cells, as shown in the small diagram. During the fourth cleavage in the formation of the sixteen cell stage (A–C, 5) d^2 is the first cell formed, which is budded off from D in a dorso-anterior direction. The division of d^1 follows immediately. Next the macromeres C, B and A with their ectomeres divide, thus producing a twelve, fourteen and sixteen cell stage respectively. The embryo is now composed of four rows with four cells each (A–C, 5). Jennings describes the sixteen-celled embryo as being composed of four layers, each layer containing a single derivative of each quadrant. The first layer at the macromere end is designated as ventral and the cells are labelled accordingly. The number of layers increase with later cleavage.

The cleavage forms of the male and the female embryos at the sixteen cell stage are indistinguishable. The shape of the embryos, the size of the cells and the direction of the various cleavages (A–C, 5) are about the same. The only evidence we have to distinguish the male embryo at this stage is the presence of the two polar bodies, or more mature embryos in the same uterus. In the fifth cleavage all of the cells except the macromere D divide in a more transverse direction: thus doubling the number of rows on the surface. The inner ends of the cleavage cells withdraw towards the exterior and produce a central cavity, which is later occupied by the large cell D. Before the fifth cleavage is completed the anterior end of the cell D is partially covered by the cleavage cell immediately in front of it. The sixth cleavage is more irregular and doubles the number of cells in each of the eight rows.

Gastrulation is a double process, while the cells are passing posteriorly over the macromere D, the cell itself is moving into the central cavity. The entire process requires about fifty minutes and can be demonstrated in the living egg. The large cell D, which is now designated by the letter E, is destined to

play a very important rôle in the future development of the individual. The derivatives of *E* give rise to most of the digestive tract, the reproductive system and a few muscle fibers. All other structures are derived from the surface cells, which form a rather deep epithelial layer around the central cell *E*.

Diagrams A–C, 6, represent late cleavage stages in which *E* has passed into the interior of the embryo and is no longer visible from the exterior. A little later stage (A–C, 7) than the preceding, shows the second cleavage of *E* as represented in an optical section from dorsal view. Diagrams A–C, 8, represent an embryo of either sex in optical section, as viewed from the left side with five large central cells derived from *E*. The small stippled space between the large cells and the outer epithelium represents the first indication of the presence of the future body cavity.

C. Differentiation of the Central Cells.

The large central cells $E^{2.1.1}$ and $E^{2.1.2}$ give rise to the reproductive organs, and the derivatives of the large central cells $E^{1.1}$, $E^{2.2.1}$ and $E^{2.2.2}$ produce most of the digestive system. The large central cells now divide very rapidly and fill the interior of the embryo (A–C, 9). These central cells as stated above become differentiated into two distinct regions, the entodermal (*st*) and the reproductive portion (*r.o.*), which shows the presence of darker granules and later gives rise to the reproductive organs proper. A few muscle fibers are derived from the central mass of cells, which are directly concerned in the movements of the reproductive organs and the stomach with the digestive glands

D. Embryonic Foot and Stomodæum.

The slight swelling at the lower posterior end (A–C, 9 ft.) marks the beginning of the foot, an embryonic structure. Later it becomes well developed in both sexes and shows the presence of two distinct toes, which no doubt persisted as a functional structure in its ancestors. The foot completely disappears before the birth of the individual. Diagrams A–C, 10 show the early stages in the formation of the stomodæum (*sto.*). The embryo is considerably curved upon itself and occasions the close proximity

of the embryonic foot and the mouth. A distinct epithelium or hypodermis is present. The stomodæum in the male embryo is less prominent (B–C, 10), than in the female. The buccal pouch and pharynx is derived from this invagination. The posterior wall of the pharynx (A–C, 11) is more prominent in the female and contributes directly to the formation of the jaws or trophi. There is no indication of the formation of trophi in the male embryos.

E. The Rôle of the Entodermal and the Reproductive Cells.

The early differentiation of the derivatives of *E* bears a striking resemblance in the two sexes (A–C, 11). Two distinct regions are readily recognized. The upper and anterior part (*st.*) becomes continuous with the walls of the pharynx, and in the female (A, 12–13), gives rise to the œsophagus, the stomach and the digestive glands. The lower and more posterior portion (*r.o.*) becomes differentiated into the vitellarium, ovary, oviduct and uterus as indicated in the A series of figures. Beginning at the point of development as indicated in the series A–C, 11 the entodermal cells (*st.*), play quite a different rôle in the two sexes. In the male embryo (B–C, 11) the cells (*st.*) fuse with the wall of the invaginated pharynx as in the female, but do not give rise to any permanent structures. Later this entodermal group of cells lose their connection with the pharyngeal wall (C, 12), and are gradually utilized as food. The reproductive portion (*ts.*) gives rise to the testis and vas-deferens (C, 14). The male embryo as indicated in series B, 11–15, with the exception of the reproductive cells, undergoes an almost complete degeneration. In B, 11 the embryo corresponds to that of the normal male (*C*, 11), but later all of the non-reproductive cells deteriorate and are utilized by the embryo. When the sperm have reached their maturity, the embryo is represented by a large sperm sac (B, 15) within the delicate body wall or hypodermis. A portion of the trochal disc and cilia often persist.

In series C, 11–15, which represents the development of the normal male as it occurs in *Asplanchnia ebbesbornii*, degeneration occurs only in the digestive system. The early development

however, is comparable to the conditions found in the female (A, 11). The cells from which the œsophagus, stomach and digestive glands are formed in the female, become independent of the pharyngeal wall in the male (C, 12–13), and persist more as a compact mass of cells, entirely free from any lumen (C, 14–15). The position of these cells within the body cavity is indicated in figures C, 11–15. This group of cells gradually becomes smaller and takes up a final position in the dorso-posterior region of the body cavity, where they are held in position by delicate muscle fibers. In extreme cases, when the male lives longer than normal, the cells completely disappear within the body cavity and no doubt serve as food. The reproductive organs are well developed (C, 11–15) and communicate with the urinogenital sinus by means of the vas deferens (C, 14). The urinogenital sinus is ciliated, which aids in the passage of the sperm. No attempt was made to represent more than a few of the muscle fibers within the body cavity. The excretory system which is well developed in the male is not shown.

The development of the digestive system reaches its greatest differentiation in the female as represented in series A. The stomach becomes a rather large strongly ciliated pouch, and communicates with the pharynx by means of a slender œsophagus, which is capable of considerable distention (A, 12–13). The stomach ends blindly. There is no indication of a rudimentary intestine in the developing embryo. The bladder (contractile vacuole) is formed by an anterior evagination of the wall of the urinogenital sinus.

Activities of the Male.

Varying degrees of degeneration are found in the males of *Asplanchnia ebbesbornii*, ranging from those without a functional digestive system, to those with all organs wanting except a large testis and the delicate hypodermis. The latter is little more than a sperm sac. In both instances however, the sperm are functional and capable of fertilizing the resting eggs. The methods of fertilization are rather unique. The normal free-swimming male is sexually mature at birth, and true intromission occurs at the urinogenital sinus (cloaca) with either kind of female. In the

female-producing female, fertilization is ineffective and the female continues to reproduce parthenogenetically. In the male-producing female, after impregnation takes place, either all resting eggs are formed or male and resting eggs. The alternating of the two kinds of eggs cocurs more frequently when few sperm are present in the oviduct.

The uterus of the male-producing female may contain embryos ranging from early cleavage to late development (Fig. 2), but if at this point impregnation occurs, yolk spherules are formed within the vitellarium and all of the following eggs formed become true resting eggs. Maturation of the male and resting eggs occurs immediately after their separation from the ovary at the end of the growth period.

The sperm of the sexually mature males before their birth (Fig. 7, *m.e.*2) may be deposited into the uterus of the parent and bring about the production of resting eggs (Fig. 7, *r.eg.*) as above. In case of the extreme degenerate males (B, 11–15 and Fig. 7, *m.e.*1) the sperm sac and the delicate body wall break down, the sperm are set free in the uterus and the production and fertilization of the resting eggs follow. Again the sperm sac (B, 15) with the delicate body wall (degenerate male) may be deposited intact into the water and later the mature sperm are set free and finally get into the uterus of the different individuals. The independent entrance of the sperm is rather a simple process, since the urinogenital sinus and the lower end of the uterus is constantly being partially everted and inverted and during these activities the sperm make their entrance into the uterus. One of the peculiar activities of the normal male is the occasional eversion of the lower end of the vas deferens and discharge of the mature sperm into the water. The entrance of the sperm into the uterus independently of the male is a common method in this particular rotifer and makes possible the fertilization of a greater number of resting eggs. Those instances in which the male embryos and the resting eggs alternate in the same uterus, are often due to the later method, where sperm enter at different intervals.

The free-swimming males are rather scarce, less than one per cent. at any time, when compared with the total number of

rotifers present in the different cultures. At the close of the active period of any one cycle, nearly all of the male-producing females, within a few hours show the presence of resting eggs within the uterus. These conditions are made possible by the independent entrance of the sperm.

In general the characteristics applying to the male rotifers

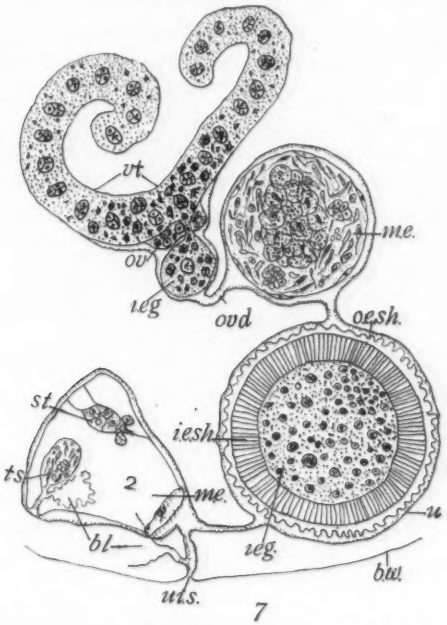

FIG. 7. Reproductive organs of a male-producing female, which contains both male embryos and resting eggs; *r.eg.*, formation of resting egg; *m.e.1.*, male embryo which shows a case of extreme degeneration, with a very delicate body wall. The central mass represents a few somatic cells that still persist. No bounding membrane of sperm sac was formed, and the sperm as development progressed were left free in the body cavity. Later the somatic cells are all utilized and the body wall becomes the bounding membrane of the large sperm sac; *m.e.2* shows a completely developed male embryo at time of birth, with testis containing mature sperm; *r.eg.*, resting egg with two distinct protective shell membranes.

as a group are the following: diminutive size, the total absence of the mouth, of the pharynx, of the jaws, of the œsophagus, of the gastric glands and of the stomach and the intestine. The

ovary is replaced by the testis. In some forms, however, like *Rhinops vitrea*, the male possesses all of the above mentioned points and does not show any indication of degeneration. In other forms as in *Polyarthra platyptera*, the male rotifer resembles a vorticella detached from its stem. Why degeneration is carried much farther in some species than in others is rather a puzzling problem. In *Asplanchnia ebbesbornii*, the varying degrees of degeneration are greater than in any previously reported species. Without the presence of the few actively free-swimming males, and more especially a knowledge of their embryonic development, the extremely degenerate males (B, 15 and Fig. 7, *m.e.*1) would in all probability remain unknown. It would not at all be surprising, if in some of the groups where males have not been reported, that extremely degenerate or parasitic males exist as described above.

COLUMBIA, MO.,
April 30, 1919.

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REFERENCE LETTERS.

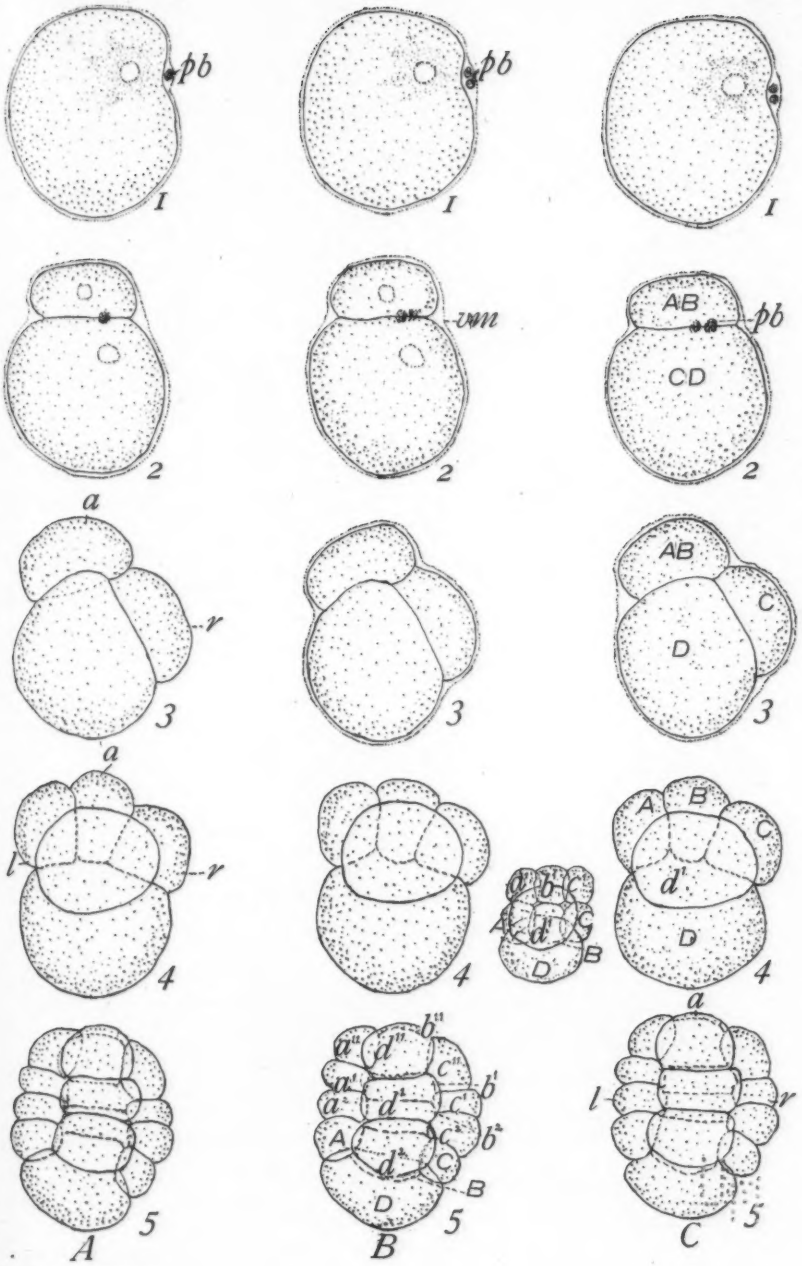
a., anterior,
b.c., body cavity,
bl., bladder,
br., brain,
bu.c., buccal cavity,
b.w., body wall,
c., cilia,
c.o., copulating organ,
cp., corpuscle,
d., dorsal,
d.g., digestive glands,
ect., ectoderm,
ent., entoderm,
fl., foot,
i.e.sh., inner egg shell membrane,
l., left,
m., muscle,
m.e., male embryo,
m.eg., male egg,
mo., mouth,
oe., œsophagus,
o.e.sh., outer egg shell membrane,
ov., ovary,
ovd., oviduct,
p., posterior,
p.b., polar body or bodies,
ph., pharynx,
phw., pharyngeal wall,
pr., proctodæum,
r., right,
r.eg., resting egg,
r.o., reproductive organs,
st., stomach,
sto., stomodæum,
ts., testis,
u., uterus,
ur.s., urino-genital sinus,
v., ventral,
v.m., vitelline membrane,
vt., vitellarium,
y., yolk.

DESCRIPTION AND EXPLANATION OF SERIES OF DIAGRAMS A, B AND C.

All drawings were made with the aid of the camera lucida, under a magnification of about one hundred and fifty diameters and reduced one half. The drawings were made from either living or mounted embryos and checked. Stippling was adopted for sake of clearness. The text figures in the A series 1–15, represent the conditions found in the female. Fig. A, 15 shows the condition of the female embryo at the time of birth. The B series 1–15, represent the male embryos that have undergone extreme degeneration. Fig. B, 15 is characteristic of the end stage of development. The C series 1–15 gives the different stages in the development of the normal male with the degenerative digestive system. The buccal pouch and pharynx disappear before birth. Fig. C, 15 represents the condition of normal male individual at the time of birth.

ERRATA.

Plates illustrating article by G. W. Tannreuther in the September issue. Should be inserted following page 208.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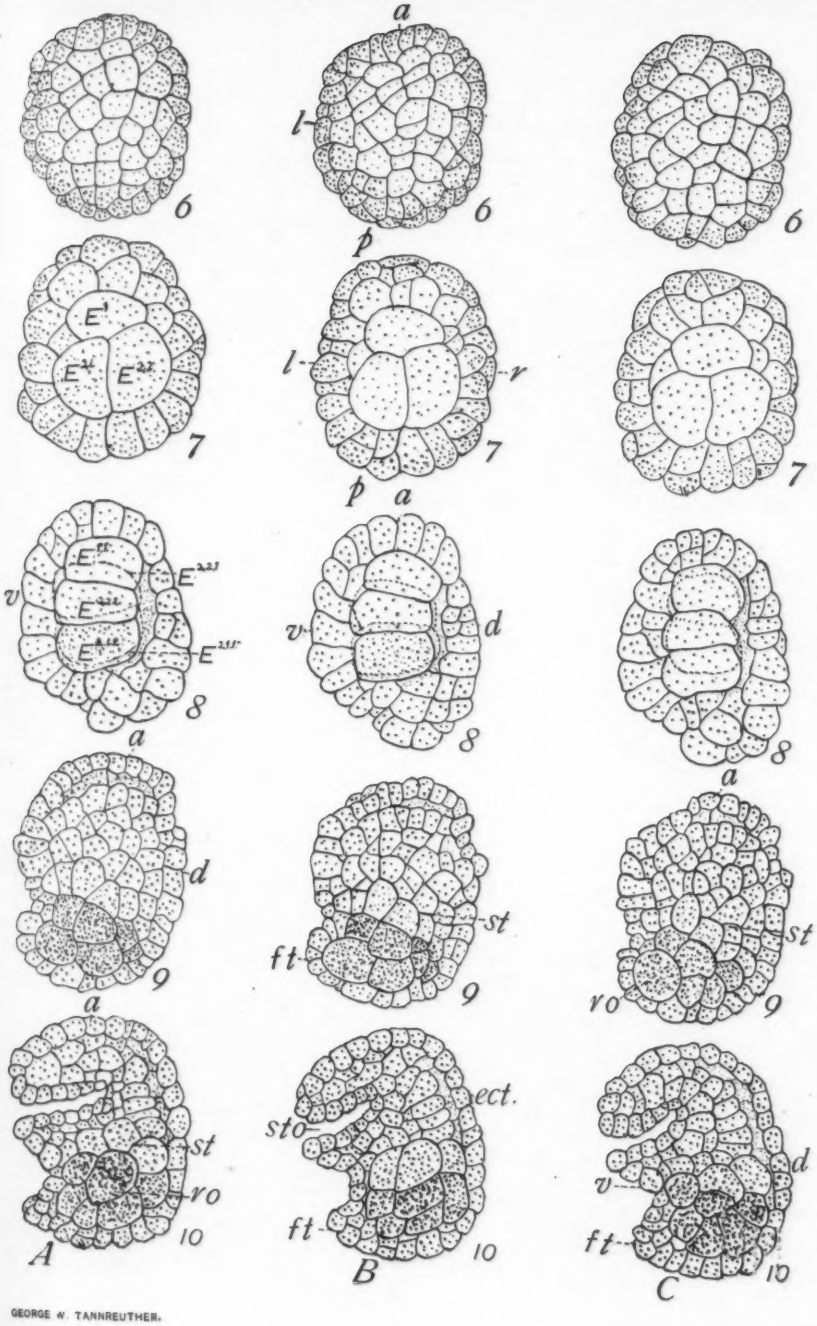
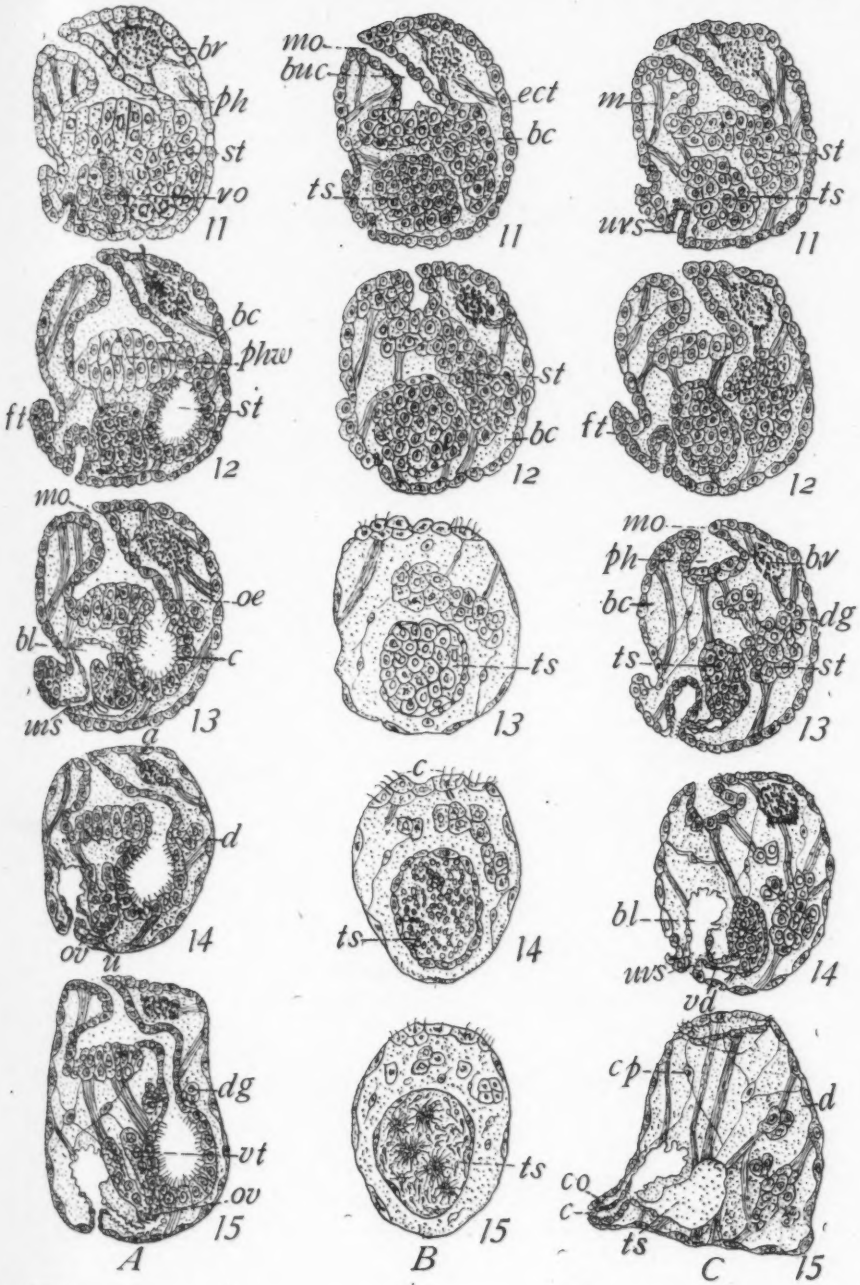


GEORGE W. TANNREUTHER.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